FORMULATION, CHARACTERIZATION AND COMPARISION OF LAMIVUDINE PRONIOSOMES PREPARED BY COACERVATION PHASE SEPARATION AND SLURRY METHOD

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI – 600 032

in partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

submitted by

Register Number: 261411264

under the guidance of

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COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

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DEPARTMENT OF PHARMACEUTICS
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DATE:

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION, CHARACTERIZATION AND COMPARISION OF LAMIVUDINE PRONIOSOMES PREPARED BY COACERVATION PHASE SEPARATION AND SLURRY METHOD "** submitted by the candidate with **Register No. 261411264** to The Tamil Nadu Dr. M.G.R. Medical University examinations is evaluated.

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Place: Chennai – 03

Date :

(Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A)

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[Prof.K.Elango, M.Pharm., (Ph.D.,)]

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LIST OF ABBREVATIONS

AIDS	- Acquired Immune Deficiency Virus
BP	- British Pharmacopoeia
FTIR	- Fourier Transform Infra Red
HAART	- Highly Active Antireteroviral Therapy
HIV	- Human Immune Deficiency Virus
IP	- Indian Pharmacopoiea
SEM	- Scanning Electron Microscopy
STD	- Sexually Transmitted Disease
UV	- Ultra Violet
WHO	- World Health Organisation
PDE	- Percentage Drug Entrapment
CPP	- Critical Packing Parameter
□ C	- Degree Celsius
MVD	- Mean Vesicle Diameter
%	- Percentage
μg	- Microgram
μm	- Micrometer
mg	- Milligram
mL	- Milliliter
mm	- Millimeter
nm	- Nonometer

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INTRODUCTION

DRUG DELIVERY SYSTEMS[1, 2]

A drug delivery system (DDS) is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. Drug delivery system is an interface between the patient and the drug. It may be a formulation of the drug to administer it for a therapeutic purpose or a device used to deliver the drug.

When a drug is taken by a patient, the resulting biological effects are determined by the pharmacological properties of the drug. These biological effects are usually produced by an interaction of the drug with specific receptors at the drug's site of action. However, unless the drug can be delivered to its site of action at a rate and concentration that both minimizes side- effects and maximizes therapeutic effects, the efficiency of the therapy is compromised. In some cases, delivery and targeting barriers may be so great as to preclude the use of an otherwise effective drug candidate.

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science, Pharmaceutics, bioconjugate chemistry, and molecular biology.[2]

NOVEL DRUG DELIVERY SYSTEM[3]

The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment.

A number of novel drug delivery system has emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity if selective uptake can be achieved. Consequently a number of vesicular drug delivery system such as liposomes, niosomes, transfersomes and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of this system that allow drug targeting and sustained or controlled release of conventional drug medicines.

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical conventional dosage forms. Even today conventional drug delivery system occupies most of the part in a prescription as well as drug store. This type of drug delivery system is known to provide a prompt release of drug. But to achieve as well as to maintain the drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day. This results in significant fluctuations in drug level.

DISADVANTAGES OFCONVENTIONAL DOSAGE FORMS[3]

i. Drugs with short half- life require frequent administration, which increases chances of missing the dose of a drug leading to poor patient compliance.
ii. A typical peak- valley plasma concentration- time profile is obtained which makes attainment of steady state condition very difficult.
iii. The unavoidable fluctuations in the drug concentration may lead to under-medication or over- medication as the C_{ss} values fall or rise beyond the therapeutic range which may lead to adverse effects of the drug.
iv. Drug accumulation may occur in case of the frequent administration of the drugs.

To overcome the above drawbacks, drug delivery systems capable of controlling the rate of drug delivery, sustaining the duration of therapeutic action and/ or targeting the drug to a particular tissue was developed.

The novel drug delivery systems are diversely referred to as "controlled release", "sustained release", "zero- order", "reservoir", "monolithic", "membrane- controlled", "smart", "stealth" etc. some of these common terms are defined as follows:

- **Prolonged/ sustained release**: the delivery system prolongs therapeutic blood or tissue levels of the drug for an extended period of time.
- **Zero order release**: the drug release does not vary with time; thus the delivery system maintains a (relatively) constant effective drug level in the body for prolonged periods.
- **Variable release**: the delivery system provides drug input at a variable rate, to match, for example endogenous circadian rhythms, or to mimic natural biorhythms.
- **Bio- responsive release**: the system modulates drug release in response to a biological stimulus (e.g. blood glucose levels triggering the release of insulin from a drug delivery device)
- **Modulated/ self regulated release**: the system delivers the necessary amount of drug under the control of the patient.
- **Rate- controlled release**: the system delivers the drug at some pre-determined rate, whether systemically or locally, for a specific period of time.
- **Targeted- drug delivery**: the delivery system achieves site- specific drug delivery.
- **Temporal- drug delivery**: the control of delivery to produce an effect in a desired time- related manner.
- **Spatial- drug delivery**: the delivery of a drug to a specific region of the body (thus this term encompasses both route of administration and drug distribution).
- **Bioavailability**: the rate and extent at which a drug is taken up into the body.
- **Repeat action**: individual dose is released fairly soon after administration and second or third doses are subsequently released at intermittent intervals.

TARGETED/ SITE- SPECIFIC DRUG DELIVERY SYSTEM [4]

While rate- controlled systems can deliver the drug at a predetermined rate, they are generally unable to control the fate of the drug, once it enters the body. Targeted drug delivery system refers to systems that place the drug at or near the receptor site or site of action. Targeted drug delivery implies selective and effective localization of drug into the target(s) at therapeutic concentrations with limited access to target sites.

Site- specific drug delivery is desirable in therapeutics, in order to improve:

- ***Drug safety***, as toxic side- effects caused by drug action at non- target sites are minimized.
- ***Drug efficacy***, as the drug is concentrated at the site of action rather than being dispersed throughout the body.
- ***Patient compliance***, as increased safety and efficacy should make therapy more acceptable and thus improve compliance.

Technologies for drug targeting are concerned with delivering drugs to specific targets in the body and also to protect drugs from degradation and premature elimination. They include the use of

- ***Soluble carriers***, such as monoclonal antibodies, dextrans, soluble synthetic polymers
- ***Particulate carriers***, such as liposomes, niosomes, micro- and nano- particles, microspheres
- ***Target- specific recognition moieties,*** such as monoclonal antibodies, carbohydrates and lecithins.

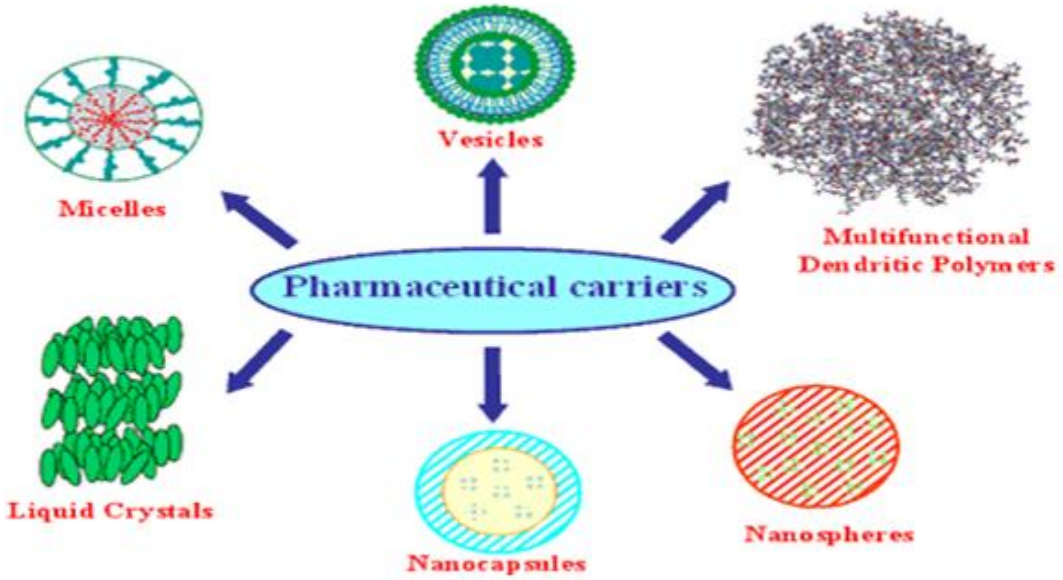
Recent advances in biological and chemical sciences have led to the development of various "smart" technologies to ensure more effective drug delivery and targeting of drugs to specific sites within the body. Such approaches include the use of:

- Antibody- directed enzyme/ prodrug therapy (ADEPT)
- Virus- directed prodrug/ enzyme therapy (VDEPT)
- Chemical drug delivery systems

DRUG DELIVERY CARRIERS [2]

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as drug delivery systems. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity. The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possesses amphiphilic and/or mesogenic properties.

Fig:1 Pharmaceutical carriers



VESICULAR DRUG DELIVERY SYSTEMS[5]

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphillic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphillic building blocks. Biologic origin of these vesicles was first reported in 1965 by 'Bingham' and has been given the name Bingham bodies.

Now a days, vesicles as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles were found to be of value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering. Vesicular delivery system provides an efficient method for delivery to the site of infection, leading to reduce of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and lipophilic drugs. Different novel approaches used for delivering the drugs by vesicular system include liposomes, niosomes, sphingosomes, transfersomes and pharmacosomes.

Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved. Lipid vesicles are one type of many experimental models of biomembranes which evolved successfully, as vehicles for controlled delivery. For the treatment of intracellular infections, conventional chemotherapy is not effective, due to limited permeation of drugs into cells. This can overcome by the use of vesicular drug delivery systems.

ADVANTAGES OF VESICULAR DRUG DELIVERY [5]

1. Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
2. Improves the bioavailability especially in the case of poorly soluble drugs.
3. Both hydrophilic and lipophilic drugs can be incorporated.
4. Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.

These vesicular systems are accompanied with some problems like drug carriers and externally triggered (eg., temperature, pH, or magnetic sensitive) carriers load drugs passively, which may lead to low drug loading efficiency and drug leakage in preparation, preservation and transport *in- vivo*. Need of intensive sonication, lead to leakages of drug during storage. Thus the major problem of their stability acts as a barrier and thus limiting their use.

The targeted vesicles are classified on the basis of their composition as

- **Lipoidal biocarriers**
  - Liposomes
  - Emulsomes
  - Enzymosomes
  - Ethosomes
  - Sphingosomes
  - Transfersomes
  - Pharmacosomes
  - Virosomes
- **Non- lipoidal biocarriers**

i. Niosomes

ii. Bilosomes

iii. Aquasomes

LIPOSOMES [9]

Liposomes or lipid based vesicles are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayered structures. The assembly into closed bilayered structures is a spontaneous process and usually needs some input of energy in the form of physical agitation, sonication, heat etc. Since lipid bilayered membrane encloses an aqueous core, both water and lipid soluble drugs can be successfully entrapped into the liposomes.

The lipid soluble or lipophilic drugs get entrapped within the bilayered membrane whereas water soluble or hydrophilic drugs get entrapped in the central aqueous core of the vesicles. For drug delivery applications liposomes are usually unilamellar and range in diameter from about 50 – 150 nm. Larger liposomes are rapidly removed from the blood circulation. Liposomes are unique in their ability

to accommodate drugs, which differ widely in physicochemical properties such as polarity, charge and size. **(vesicular stability issues)**

NIOSOMES [5]

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nanometric scale. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphillic and lipophillic drugs. Although structurally similar to liposomes, they offer several advantages over them.

Advantages

- Better patient compliance and better therapeutic effect in comparison to oily formulations. Can be used to deliver hydrophilic, lipophilic as well as amphiphilic drugs and can accommodate drugs with wide range of solubility.
- Controlled and sustained release of drugs due to depot formation. Enhance the oral bioavailability of drugs.
- Osmotically active and stable.
- Biocompatible, biodegradable, non-toxic and non-immunogenic. Protect the drug from enzymatic metabolism thus increases the stability of drug
- Drug targeting to various organs.
- Enhance the skin permeation of drugs.
- Easy to handle, store and transport.
- Administered by various routes via oral, parenteral, topical etc.
- The shape, size, composition and fluidity of niosomes can be controlled as and when required.

PHARMACOSOMES [5]

These are defined as colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates, depending on the chemical structure of drug-lipid complex15. The prodrug conjoins hydrophilic and lipophilic properties and therefore acquires amphiphillic

characters and was found to reduce interfacial tension and thus at higher concentrations exhibits mesomorphic behavior. Because the system is formed by linking a drug (pharmakon) to a carrier (soma), they are called pharmacosomes. Pharmacosomes bearing unique advantages over liposome and niosome vesicles have come up as potential alternative to conventional vesicles.

ETHOSOMES [9]

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatitidic acid), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization therefore, when integrated into a vesicle membrane it gives that vesicle the ability to penetrate the stratum corneum. Also because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.

TRANSFERSOMES [9]

Transfersomes are specially optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact. Each transfersome consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of **"edge activators"** into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxycholate, span 80 and Tween 80, have been used as edge activators. It was suggested that transfersomes could respond to external stress by rapid shape transformations requiring low energy. These novel carriers are applied in the form of semi-dilute suspension, without occlusion. Due to their deformability, transfersomes are good candidates for the non-invasive delivery of small, medium and large sized drugs. They have been used as drug carriers for a range of small molecules, peptides, proteins and vaccines, both in vitro and in vivo.

Materials commonly used for the preparation of transfersomes are phospholipids (soya phosphatidyl choline, egg phosphatidyl choline), surfactant (tween 80, sodium cholate) for providing flexibility, alcohol (ethanol, methanol) as a solvent, dye for confocal scanning laser microscopy (CSLM) and buffering agent (saline phosphate buffer pH 7.4), as a hydrating medium.

COLLOIDOSOMES [9]

Colloidosomes are the hollow shell microcapsules consisting of coagulated or fused particles at interface of emulsion droplets. Colloidosomes have exciting potential applications in controlled release of drugs, proteins, vitamins as well as in cosmetics and food supplements. Colloidosomes have a great encapsulation efficacy with a wide control over size, permeability, mechanical strength and compatibility. Colloidosomes assemble polymer latex colloidal particles into shells around water-in-oil emulsion drops followed by partial fusion of shell and centrifugal transfer into water to yield stable capsules in which the shell permeability can be controlled by adjustment of partial fusion conditions. Hairy colloidosomes whose shell consists of microrod particles, are designed and fabricated novel colloidosome capsules that consist of aqueous gel core and shells of polymeric microrods. This has been achieved by templating water-in-oil emulsions stabilized by rod like particles followed by gelling of the aqueous phase, dissolution of oil phase in ethanol and redispersion of obtained colloidosome microcapsules in water.

HERBOSOMES [9]

The term "herbo" means plant, while "some" means celllike. Over the past century, phyto-chemical and phyto-pharmacological sciences established the compositions, biological activities and health promoting benefits of numerous botanical products. Most of the biologically active constituents of plants are polar or water soluble molecules. However, water soluble phytoconstituents (like flavonoids, tannins, glycosidic aglycones etc) are poorly absorbed either due to their large moleculer size which cannot be absorbed by passive diffusion, or due to their poor lipid solubility, severely limiting their ability to pass across the lipidrich biological membranes, resulting in poor bioavailability. Phytomedicines, complex chemical mixtures prepared from plants, have been used for health

maintenance since ancient times. But many phytomedicines are limited in their effectiveness because they are poorly absorbed when taken by mouth. Herbosomes are also often known as phytosomes. Molecular layer consisting of PC and other phospholipids provides a continuous matrix into which the proteins insert.

SPHINGOSOMES [9]

Sphingosomes are concentric, bilayered vesicle in which an aqueous volume is enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipids. Sphingosomes are comprised of sphingolipids and cholesterol, an interior aqueous environment having pH less than that of exterior. The drug is encapsulated inside the lipid bilayer and is delivered to the host at a predetermined rate thereby improving the efficacy, increasing the circulation time and reducing the toxicity. Sphingosomes can be utilized for therapeutic, cosmetic and diagnostic purpose for the delivery of active to the target site or organ.

They can be administered by variety of routes like oral, parenteral, inhalation, transdermal etc. Sphingolipid present in the sphingosomes offer several advantages to these vesicular systems for targeting both by passive and active targeting mechanism.

CUBOSOMES [9]

Bicontinuous cubic liquid crystalline materials are active ingredients because they give the unique structural ends to controlled release applications. Amphiphillic molecules form bicontinuous water and oil channels, where "bicontinuous" refers to two distinct (continuous, but non-intersecting) hydrophilic regions separated by the bilayer. Cubosomes are discrete, sub micron, nanostructured particles of bicontinuous cubic liquid crystalline phase. Cubosomes possess the same microstructure as the parent cubic phase but have much larger specific surface area and their dispersions have much lower viscosity than the bulk cubic phase. The ability of cubic phases to exist as discrete dispersed colloidal particles or cubosomes is perhaps the most intriguing. Whereas most concentrated surfactants that form cubic liquid crystals lose these phases to micelle formation at high dilutions, a few surfactants have optimal water

insolubility. Their cubic phases exist in equilibrium with excess water and can be dispersed to form cubosomes.

LAYEROSOMES [9]

The layer-by-layer coating concept is one of the strategies used for the preparation or the stabilization of nanosystems. The layersomes are conventional liposomes coated with one or multiple layers of biocompatible polyelectrolytes in order to stabilise their structure. The formulation strategy is based on an alternative coating procedure of positive poly (lysine) (pLL) and negative poly (glutamic acid) (pGA) polypeptides on initially charged small unilamellar liposomes. Oral administration or their incorporation in biomaterials is among potential fields of application.

UFASOMES [9]

The fatty acid vesicles are named "ufasomes," ufosomes are unsaturated fatty acid liposomes. Fatty acid vesicles are colloidal suspensions of closed lipid bilayers that are composed of fatty acids and their ionized species (soap). They are observed in a small region within the fatty acid-soap-water ternary phase diagram above the chain melting temperature (Tm) of the corresponding fatty acid-soap mixture. Fatty acid vesicles always contain two types of amphiphiles, the nonionized neutral form and the ionized form (the negatively charged soap). The ratio of nonionized neutral form and the ionized form is critical for the vesicle stability. Fatty acid vesicles are actually mixed "fatty acid/soap vesicles". Ufasome membranes are much more stabilized in comparison to liposomes.

CRYPTOSOMES [11]

Stealth liposomes, also known as immune-liposomes or cryptosomes are liposomes that evade detection in an immune system. Stealth liposomes or cryptosomes are designed to circulate for longer periods of time *in- vivo*, but they are different from "long-circulating liposomes". Stealth liposomes use polyethylene glycol (PEG) as a steric stabilizer. The properties of the stealth liposome depend on the way PEG is linked to the lipids. It is important to note that stealth liposomes are not fully inert vesicles; they can eventually become detected by the immune system. It could be used for slow release of drug or for imaging purposes. Incorporation of polymers, such as polyethylene glycol-lipid

derivatives, or glycolipids into liposomes results in sterically stabilized liposomes which have several advantages over liposome formulations traditionally used in the past, including reduced recognition and uptake by macrophages, extended circulation half-lives, targeted drug delivery dose-independent pharmacokinetics, and increased uptake *in- vivo* by solid tumors, breast cancer.

ULTRASOMES [11]

Ultrasomes are specialized liposomes encapsulating an endonuclease enzyme extracted from *Micrococcus luteus*. Endonuclease recognizes ultra violet (UV) damage and is reported to accelerate its repair four-folds. Ultrasomes also protect the immune system by repairing UV-DNA damage and reducing the expression of tumor necrosis factor (TNF-α), interleukins (IL-1, IL-6 and IL-8). They stimulate the production of melanin by melanocytes in the tanning response following UV exposure and are used in cosmeceuticals and anti-aging formulations.

PHOTOSOMES[11]

These are artificial spherical submicroscopic vesicles with diameter between 25 and 5000 nm. Photosomes are composed of amphiphilic molecules with core that consists of an aqueous cavity, which is encapsulated by one or more bimolecular phospholipid sheets separated from each other by aqueous layers. Photosomes contain the enzyme photolysase encapsulated in a liposome structure and are incorporated in sun-care product to protect the sun exposed skin by releasing a photo-reactivating enzyme extracted from a marine plant, *Anacystis nidulans*. This enzyme can be activated by light and can work during the day to support the skin deoxyribonucleic acid (DNA) repair process.

Combined with ultrasomes, they constitute the "intelligent" DNA repair system and are the most widely used in cosmetic delivery systems and photodynamic therapy.

EMULSOME[11]

Emulsomes are a new generation of colloidal carrier systems in which internal core is made of fats and triglycerides which is stabilized by high concentration of lecithin in the form of o/w emulsion. Emulsomes have the characteristics of both liposomes and emulsions. The solidified or semi-solidified internal oil core provides a better opportunity to load lipophilic drugs in high concentrations. Simultaneously, a prolonged controlled release can also be expected and the ability to encapsulate water-soluble medicaments in the aqueous compartments of surrounding phospholipid layers. The solvent free and surfactant free emulsomes technology have demonstrated high drug en-capsulation capacity for water insoluble antifungal and anticancer drugs showing enhanced drug delivery and improved preclinical efficacy for parenteral routes.

GENOSOMES[11]

Genosomes are complex of genetic material like DNA and suitable lipid. They are also known as lipoplexes that are used to deliver genes. They are artificial macromolecular complexes for functional gene transfer. Cationic lipids are most suitable for this delivery system because they possess high biodegradability and stability in the blood stream. Mostly DNA-cationic liposome complexes were used to translocate DNA across cellular membranes *in- vivo*, because interaction between DNA-lipid membranes has proved crucial to the understanding of the colloidal state of the genosomes. These DNA lipid complexes could be later aggregated into higher order assemblies, creating stacked lipid-DNA multilayers, for generating more protection.

VIROSOMES[11]

Virosomes are reconstituted viral envelopes including membrane lipids and viral spike glycoproteins but devoid of viral genetic material. The external surface of the virosome resembles that of a virus particle with spike proteins protruding from the membrane but their interior compartment is empty. Virosomes were first prepared with inserted purified influenza spike proteins into preformed liposomes. Thereafter a range of viral envelopes have been reconstituted including those of Sendai virus, Semliki Forest Virus (SFV), Vesicular Stomatitis Virus (VSV) and Sindbis virus. Because virosomes display

viral envelope glycoproteins which in their native conformation stimulate humoral responses, they are highly effective as vaccine antigens and adjuvants.

ENZYMOSOME[11]

The structure in which an enzyme is enclosed in a liposome is called an enzymosome. These are liposomal constructs engineered to provide a mini bioenvironment in which enzymes are covalently immobilized or coupled to the surface of the liposomes thereby targeting the drugs to the tumor cells.

VESOSOME[11]

Vesosomes are multicompartment structures which has distinct inner compartments separated from the external membrane. Each compartment of vesosome can encapsulate different materials and have different bilayer composition. Vesosome could entrap both colloidal particles and bio-logical macromolecules relatively efficiently. While small molecules are released from unilamellar liposomes in minutes, they are retained in vesosomes from hours to days, even though the liposomes and vesosomes have the same bilayer composition and size. Vesosomes are formed by adding ethanol to a variety of saturated phospholipids. At temperatures below the gel-liquid crystalline transition, Tm, the inter digitated lipid-ethanol sheets are rigid and flat; when the temperature is raised above Tm, the sheets become flexible and close on themselves and the surrounding solution to form closed compartments.

SUBTILOSOMES[11]

Subtilosomes are prepared from phospholipids isolated from *Bacillus subtilis*. They are novel potential carrier system used in drug delivery. Cardiolipin and phosphatidyl glycerol are abundant in *B. subtilis*.

ESCHERIOSOMES[11]

These are lipoidal vesicles, prepared from polar lipids extracted from *Escherichia coli*. Majorly phosphatidyl ethanolamine, cardiolipin, and phosphatidyl glycerol are classes of phospholipid, present in *E. coli*. Escheriosomes encapsulated antigen elicit strong humoral immune response in immunized animals, in general, escheriosomes are considered as potential

candidate vaccine carrier system capable of eliciting both cell-mediated as well as humoral immune responses.

MARINOSOMES[11]

Marinosomes are liposomes based on a natural marine lipid extract containing high ratio of polyunsaturated fatty acids (PUFA) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are not present in normal skin epidermis. They are metabolized by skin epidermal enzymes into anti-inflammatory and anti-proliferative metabolites that are beneficial in treating inflammatory skin disorders. However, the preventing effect of marinosomes was highly dependent on the lipid concentration used and the liposome mean diameter. Active and passive loading of drug, as well as complex structural rearrangements directly depends on transmembrane pH gradient. All these results allowed considering marinosomes as potential candidates for cosmeceutical and oral PUFA supplements in view of the prevention and treatment of deficiencies.

ASPASOMES[11]

Vesicles prepared with amphiphiles having antioxidant property may have potential applications towards disorders implicated with reactive oxygen species. Ascorbyl palmitate (ASP) was explored as bilayered vesicle forming material. It forms vesicles in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Aspasome rendered much better antioxidant activity.

ARCHAEOSOME[11]

Archaeosome constitute a novel family of liposome made with one or more of the fully saturated bipolar tetra ether lipids, which exerts a higher stability in comparison with conventional lipids to several conditions such as high temperature, alkaline or acidic pH, and presence of phospholipases, bile salts and serum media. They are nano-sized vesicles prepared from total polar lipids either extracted from the selected genera and species of the Archaea domain or synthetic archaeal lipids. Incorporation of polyethylene glycol and coenzyme Q10 into archaeosomes has been found to alter the tissue distribution profiles of intravenously administered vesicles. Also, intravenous and oral delivery of nanometric-sized archaeosomes to an animal model was well tolerated with no apparent toxicity.

DISCOMES [9]

Niosomes are solubilised with Sollulan C24 (polyoxy ethylene cetyl ether class) in order to effect vesicle to discome transition. Discomes are relatively large in size (12- 60 micrometer) and are capable of entrapping high quantities of water-soluble solutes. They were found to release the contents following biphasic profile particularly in the case where the drug was loaded using pH gradient technique. The prepared system could produce or sustain a suitable activity profile upon administration. The discomes were found to be promising and of potential for controlled ocular administration of water- soluble drugs.

BILOSOMES [5]

Bilosomes are the novel innovative drug delivery carriers consist of deoxycholic acid incorporated into the membrane of niosomes. As conventional vesicles (liposomes and niosomes) can cause dissolution and undergo enzymatic degradation in gastro intestinal tract but incorporation of bile salts (commonly used penetration enhancers) in niosomal formulation could stabilize the membrane against the detrimental effects of bile acids in GI tract. These bile salt stabilized vesicles are known as bilosomes. These are highly biocompatible and have been found to improve the therapeutic efficacy of drugs due to their stability in gastro intestinal tract. Bilosomes have been found to increase the bioavailability of drugs as they can readily absorbed through small intestine to the portal circulation (hepatocirculation). Through this circulation they approach to liver and release the drug, so found to be an effective tool in drug targeting to liver.

AQUASOMES [5]

Molecules are adsorbed by copolymerization, diffusion or adsorption with or without modification. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. Aquasomes are spherical 60- 300nm size particles called “bodies of water”. Due to their size and structural stability, these avoid clearance by reticuloendothelial system and degradation by other environmental changes.

HEMOSOMES[11]

New artificial oxygen transporting systems engineered by immobilizing haemoglobin with polymerisable phospholipids, capable of prolonged activity in the circulation are of special interest. Natural hemoglobin was incorporated into liposomes of different composition (so- called hemosome). It was shown that the maximal quantity of hemoglobin obtained from lysed erythrocytes incorporates into negatively charged liposomes. Stabilized hemosomes bind oxygen in the same way as human blood hemolysates.

APPROACHES FOR IMPROVEMENT OF VESICULAR SYSTEM[4]

Pro-vesicular Drug Delivery

Pro-vesicular drug delivery developed to overcome the stability problems associated with vesicular drug delivery systems composed of water soluble porous powder as a carrier drug is dissolved in organic solvent to produce free-flowing granular product. It can avoid many of the problems associated with aqueous vesicular dispersions.

Types of pro vesicular drug delivery systems

- Proliposomes,
- Proniosomes

PROLIPOSOMES

Comparision between liposomes and proliposomes. Liposomes-Unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids. They show controlled release and increased solubility. But have tendency to aggregate or fuse, susceptible to hydrolysis or oxidation. Proliposomes- an alternative forms to conventional liposomal formulation Composed of water soluble porous powder as a carrier, phospholipids and drugs dissolved in organic solvent. Lipid and drug are coated on to a soluble carrier to form free-flowing granular material. Show controlled release, better stability, ease of handling and increased solubility.

PRONIOSOMES [24,26]

Proniosomes are non ionic surfactant-coated carrier, which when needed, are rehydrated by brief agitation in water. These are considered superior drug delivery system because of their low cost, greater stability, non-toxicity, biocompatible, biodegradable and non-immunogenic, as it is nonionic in nature.

ADVANTAGES OF PRONIOSOMES

Liposomes and niosomes are well known drug/ cosmetic delivery systems. But these delivery systems have been reported to have many disadvantages in terms of preparation, storage, sterilization, etc. the disadvantages of liposomes and niosomes are given below, which can be overcome by Proniosomes.

- Liposomes and niosomes are dispersed aqueous system and have a problem of degradation by hydrolysis or degradation.
- Liposomes and niosomes require special storage and handling.
- Sedimentation, Aggregation or fusion on storage is usually seen.
- In liposomes, purity of natural phospholipids is also variable.
- Difficulty in sterilization, transportation, distribution, storage uniformity of dose and scale up.
- Use of unacceptable solvents.
- Incomplete hydration of the lipid / surfactant film on the walls during hydration process.

Disadvantages of Niosomes

- Physical instability
- Aggregation
- Fusion
- Leaking of entrapped drug
- Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

Table 1 - Commonly used materials for Proniosome preparation

S.No	Class	Examples	Use
1.	Surfactants	Span 20,40,60,80,85,Tween 20,40,60,80.	To increase drug flux rate cross the skin.
2.	Cholesterol	Cholesterol	To prevent the leakage of drug formulation.
3.	Lecithin	Lecithin	Penetration enhancer.
4.	Maltodextrin	Maltodextrin	Provides flexibility in surfactant and other component ratio.
5.	Sorbitol	Sorbitol	Alters the drug distribution.

STRUCTURE OF NIOSOMES

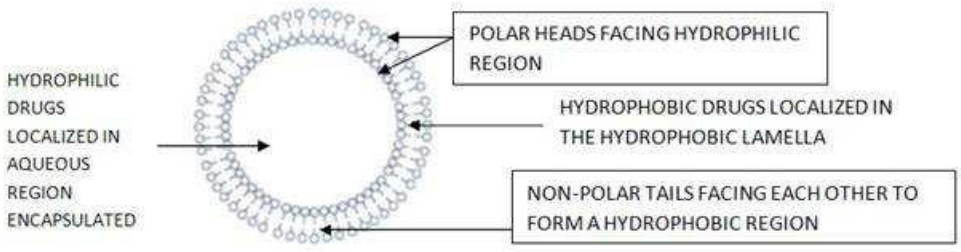


Figure:2 structure of niosomes

These are microscopic lamellar structure. They combine a non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class of cholesterol followed by hydration in the aqueous media. The surfactant molecule direct themselves such that the

hydrophilic end of the non- ionic surfactant orient outward, while the hydrophobic end are in the opposite direction to form the bilayer. Like liposomes proniosomes are also made of bilayer. In proniosomes this bilayer are made up of non-ionic surface active agent. On the basis of method of preparation proniosomes are unilamellar or multilamellar.

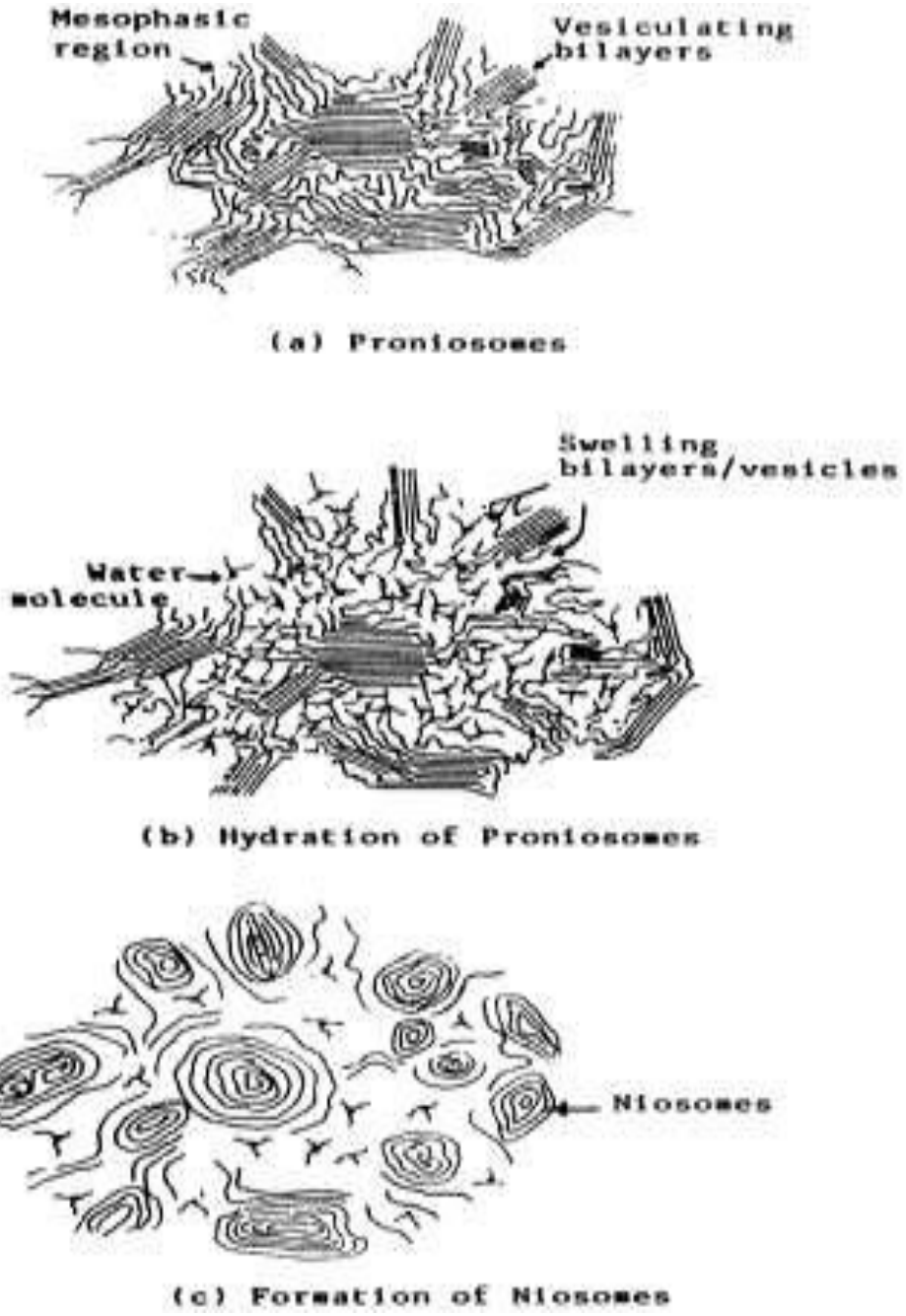


Figure:3 Formation of niosomes from proniosomes

TYPES OF PRONIOSOMES [13,17,24]

1) Dry granular proniosomes

- **Sorbital based proniosomes**
- **Maltodextrin based proniosomes**

2) Liquid crystalline proniosomes.

Sorbitol based proniosomes

Sorbital based proniosomes are is a dry formulation which consist proniosomes as carrier, which is further coated with non-ionic surfactant and is used as niosomes within a minute by addition of hot water followed by agitation. This are usually made by spraying the surfactant mixture prepared in organic solvent on to the sorbitol powder then evaporating the solvent. Since the sorbitol carrier is soluble in organic solvent, the process is required to be repeated till the desired surfactant coating has been achieved. In sorbitol based proniosomes size distribution is uniform. It is useful in case where the active ingredient is susceptible to hydrolysis. The residual sorbitol decreases entrapment efficiency to less than one half of that observed with sorbitol. This necessiate reduction in proportion of carrier in final niosome suspension. The difficulty lies in testing of sorbitol particles because sorbitol is soluble in chloroform and other organic solvent. Its prepared by slow spraying method.

Maltodextrin based proniosomes

It is prepared by fast slurry method. Time required to produce proniosomes by slurry method is independent of the ratio of surfactant solution to carry out. Proniosomes of high surface to carrier ratio can be carried out. Proniosomes of high surface to carrier ratio can be prepared. The method of obtaining niosomes from such a proniosomes for the drug delivery is very simple. An analogue process with Sorbital results in a solid, surfactant/sorbitol cake. Since maltodextrin morphology is preserved, hollow blown maltodextrin particles can be used for significant gain in surface area. The higher surface area results in thinner surface coating, which makes

the rehydration process efficient. This preparation has potential of application in delivering of hydrophobic and amphiphilic drug molecule.

Liquid crystalline proniosomes

When the surfactant molecule is kept in contact with water, there are three ways through which lipophilic chains of surfactant can be transformed into a disordered, liquid state called lyotrpoic liquid crystalline state(neat phase). These three ways are increasing the Kraft temperature (Tc), addition of solvent which dissolve lipids, and use of both temperature and solvent. Neat phase also known as lamellar phase contains bilayer arranged in a sheet over one another within intervening aqueous layer. This type of structure gives typical x-ray diffraction and thread like birefrigerant structure under polarized microscope. The lamellar crystalline phase is converted into niosomes at higher concentration. The liquid crystalline proniosomes and proniosomal gel acts as reservoir for transdermal drug delivery.

Liquid crystalline proniosomes display a number of advantages:

- Stability
- High entrapment efficiency
- As a penetration enhancer
- Easy to scale up as no lengthy process is involved; moreover it avoids the use of pharmaceutically unacceptable additives.

COMPONENTS OF PRONIOSOMES[24]

The essential components of the delivery system are as follows

Surfactants

Surfactants are the surface active agent usually organic compounds that are amphiphilic in nature (having both hydrophobic and hydrophilic groups). They have variety of functions including acting as solubilizers, wetting agents, emulsifiers and permeability enhancer. The most common non-ionic amphiphiles used for vesicle formation are alkyl ethers, alkyl esters, alkyl amides and esters of fatty acids. Selection of surfactant should be done on the basis of HLB value. As Hydrophilic Lipophilic Balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. The water soluble detergent polysorbate 20 also forms niosomes in the presence of cholesterol. This is despite the fact that the HLB number of this compound is 16.7 Degree of entrapment is affected by the HLB of a surfactant. Transition temperature of surfactants also affects the entrapment of drug in vesicles. Spans with highest phase transition temperature provide the highest entrapment for the drug and vice versa. Span 40 and Span 60 produces vesicles of larger size with higher entrapment of drug. The drug leaching from the vesicles is reduced due to high phase transition temperature and low permeability. High HLB value of Span 40 and 60 results reduction in surface free energy which allows forming vesicles of larger size hence large area exposed to the dissolution medium and skin. No significant difference is observed in the skin permeation profile of formulation containing Span 60 and Span 40surfactant, due to their higher phase transition temperature that is responsible for their lower permeability. The encapsulation efficiency of Tween is relatively low as compared to Span.

The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of Surfactants can predicate geometry of vesicle to be formed.

Critical packing parameters can be defined using following equation,

$$CPP = \frac{V}{Lc \times Ao}$$

CPP = 0.5 – 1 spherical vesicles form

CPP = 1 inverted vesicles form

V = Hydrophobic group volume

Lc = the critical hydrophobic group length,,

Ao = the area of hydrophilic head group.

Table2: List of surfactants used in proniosomes formulation

Non-ionic amphiphiles	Examples
Alkyl ethers and alkyl glyceryl Ethers	Polyoxyethylene 4 lauryl ether (Brij30)
Polyoxyethylene cetyl ethers	Brij 52, 56, 58
Polyoxyethylene stearyl ethers	Brij 72, 76
Sorbitan fatty acid esters	Span 20, 40, 60, 80
Polyoxyethylene sorbitan fatty acid esters	Tween 20, 40, 60, 80

Carrier material

The carrier when used in the proniosomes preparation permits the flexibility in the ratio of surfactant and other components that incorporated. In addition to this, it increases the surface area and hence efficient loading. The carriers should be safe and non-toxic, free flowing, poor solubility in the loaded mixture solution and good water solubility for ease of hydration. Commonly used carriers are listed, they are sorbitol, mannitol, Glucose, Lactose, Sucrose stereate. Another polysaccharide that can be used as carrier is maltodextrin .It has minimal solubility in organic solvent. Thus it is possible to coat maltodextrin. It forms particles by simply adding surfactant in organic solvent. The use of maltodextrin as carrier in Proniosomes preparation permitted flexibility in the ratio of surfactant and other components which can be incorporated.

Membrane stabilizers

Cholesterol and lecithin are mainly used as membrane stabilizer. Steroids are important components of cell membrane and their presence in membrane and their presence in membrane brings about significance changes with regard to bilayer stability, fluidity and permeability. Cholesterol is a naturally occurring steroid used as membrane additive. It prevents aggregation by the inclusion of molecules that stabilize the system against the formation of aggregate by repulsive steric or electrostatic effects. It leads transition from the gel state to liquid phase in niosomes system. Phosphatidylcholine is a major component of lecithin. It has low solubility in water and can form liposomes, bilayer sheets, micelles or lamellar structures depending on hydration and temperature. Depending upon the source from which they are obtained they are as named as egg lecithin and soya lecithin. It acts as stabilizing as well as penetration enhancer. It is found those vesicles composed of soyalecithin are of larger size than vesicle composed of egg lecithin probably due to the difference in the intrinsic composition. Cholesterol increases or decreases the percentage encapsulation efficiency depending on either the type of the surfactant or its concentration within the formula. Cholesterol along with the addition of surfactant forms homogenous noisome dispersion rather than only a surfactant which forms a gel. Cholesterol is thus usually included in a 1:1 molar ratio in most formulations as it is known to abolish the gel to liquid phase transition of niosomes systems resulting in niosomes that are less leaky. The amount of cholesterol to be added depends on the HLB value of the surfactants. As the HLB value increases above 10 it is necessary to increase the minimum amount of cholesterol to be added in order to compensate for the larger head groups. It was found that above a certain of cholesterol, entrapment efficiency decreased possibly due to a decrease in volume diameter.

Solvent and aqueous phase

Alcohol used in Proniosomes has a great effect on vesicle size and drug permeation rate. Vesicles formed from different alcohols are of different size and they follow the order: Ethanol > Propanol > Butanol > Isopropanol. Ethanol has greater solubility in water hence leads to formation of highest size of vesicles instead of Isopropanol which forms smallest size of vesicle due to branched chain present. Phosphate buffer pH 7.4, 0.1% glycerol, hot water is used as aqueous phase in preparation of proniosomes.

Drug

The drug selection criteria could be based on the following assumptions.

- Low aqueous solubility of drugs.
- High dosage frequency of drugs.
- Short half-life.
- Controlled drug delivery suitable drugs.
- Higher adverse drug reaction drugs.

Hydration medium

Phosphate buffer having various pH are most widely used hydration medium for preparationof proniosomes derived niosomes. The solubility of drug being encapsulated determines the actual pH of hydration medium. The temperature of hydration also plays an important role in governing the self-assembly of non-ionic surfactant into vesicles and affects their shape and size. In case of proniosomal gel preparation, the hydrating temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system.

Nature of encapsulated drug

The main factor in the consideration is the influence of an amphiphilic drug on vesicle formation. When drug was encapsulated in niosomes, aggregation occurred and was overcome by the addition of a steric stabilizer. When more drugs is added the increase in its encapsulation could be the result of saturation of the medium. This suggests that the solubility of certain poorly soluble drugs can be increased by formulation in niosomes but only up to a certain limit above which drug precipitation will occur. Increase in drug concentration showed an increase in both percentage encapsulation efficiency and the amount of drug encapsulated per mole total lipids upon hydration and formation of niosomes.

Formation of niosomes from proniosomes

The niosomes can be prepared from the proniosomes (as shown in figure 2) by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

$$T > Tm$$

Where,

T = Temperature

Tm = Mean phase transition temperature.

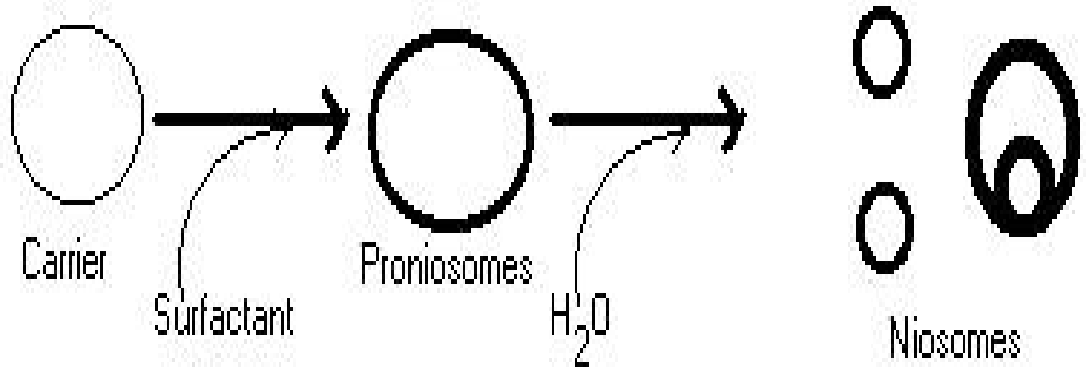


Figure 4: Formation of niosomes from proniosomes

Mechanism of Action

The exact mechanism of penetration of drug in the vesicles through the skin are not yet explored, but the penetration will depends on the nature and type of the drug used, vesicles formed and hydration temperature for the conversion of proniosomes to Niosomes. The lipids used in the preparation of proniosomes, act as carrier that will form depot at the site of action and hence sustains the action. The rate-limiting step in the penetration of drug through the transdermal drug delivery is the lipid (ceramides) part of stratum corneum, which packed tightly as bilayer by hydrogen bonding. The hydrogen bonding will strengthen and stabilize the lipid bilayer and as a result will

impart the barrier property of stratum corneum .Proniosomes will hydrate to niosomes when applied to skin. On to the skin surface, the niosomes formed adsorbs fuses and loosens the ceramides by competitively breaking the hydrogen bond network leading to high thermodynamic activity at the interface. This will increase the increases the concentration gradient and hence increases the diffusion pressure for the driving of drug through the stratum corneum.

METHOD OF PREPARATION OF PRONIOSOMES[13,17,24]

Proniosomes are prepared by following methods.

- **a.Slurry Method**
- **b.Coacervation phase separation method**
- **c.Spray coating method**.

SLURRY METHOD

Proniosomes can be prepared by addition of the carrier and the entire surfactant solution in a round bottomed flask which is fitted to rotary flash evaporator and vacuum was applied to form a dry and free flowing powder. Finally, the formulation should be stored in tightly closed container under refrigeration in light. The time required for proniosomes production is independent of the ratio of surfactant solution to carrier material and appears to be stable. The proniosomal powder formed is collected and sealed in containers and stored at 4°C.

Proniosomes are prepared by developed slurry method using Maltodextrin as a carrier. The required volume of surfactant and cholesterol stock solution per gram of carrier and drug should be dissolved in the solvent in 100 ml round bottom flask containing the carrier (Maltodextrin). Additional chloroform can be added to form slurry in case of lower surfactant loading. The flask has to be attached to a rotary flash evaporator to evaporator solvent at 50-60 rpm at a temperature of45-2□ C and a reduced pressure of 600mm Hg until the mass in the flask had become container under refrigeration in light.

Advantages of slurry method

a) Maltodextrin like polysaccharide which is easily soluble in water and it is used as carrier material in formulation; they were easily coated by simply adding surfactant in organic solvent to dry maltodextrin.

b) Due to uniform coating on the carrier it protect the active ingredient and the surfactants from hydrolysis and oxidation.

c) The higher surface area results in thinner surfactant coating which makes the rehydration process efficient.

Disadvantages of slurry method

a) Method is time consuming and involves specialised equipment with vacuum.
b) The thin film approach allows only for a predetermined lot sizes so material often wasted, so small quantities and small dose batch can be tedious one.

COACERVATION PHASE SEPARATION METHOD

Proniosomal gels can be prepared by this method which comprises of surfactant, lipid and drug in a wide mouthed glass vial along with small amount of alcohol in it. The mixture is warmed over water bath at 60-70 □C for 5min until the surfactant mixture is dissolved completely. Then the little aqueous phase is added to the above vial and warmed still a clear solution is formed which is then converted into proniosomal gel on cooling. After hydration of proniosomes they are converted to uniformly sized niosomes.

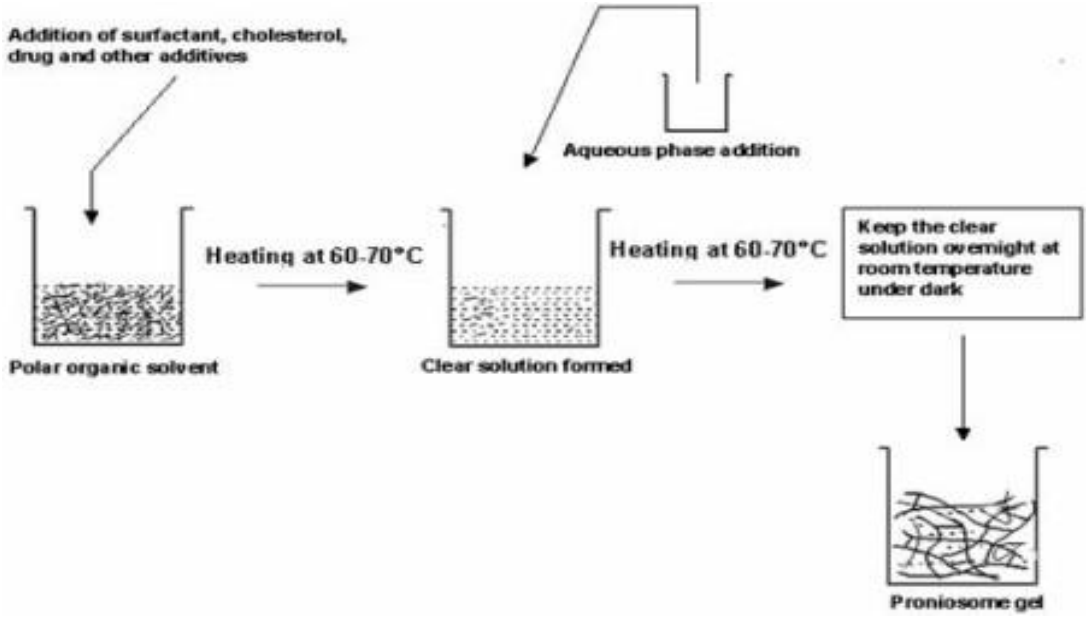


Figure 5: Diagrammatic representation of preparation of proniosomal gel

Advantages of this method

a) Method is simple and without time consumable so it does required any specialized equipment.

b) Specially adopted for gel preparation

c) Small quantities or small dose formulation can be prepared on lab scale.

SLOW SPRAY COATING METHOD

In this method, the surfactant is added to an organic solvent and sprayed onto carrier. Then the solvent is evaporated. This process is repeated until the desired surfactant loading is achieved, because the carrier is soluble in the organic solvent. As the carrier dissolved, hydration of this coating allows the formation of multilamellar vesicles. These niosomes have uniform size distribution and similar to those produced by conventional methods. A 100 ml round bottom flask containing desired amount of carrier can be attached to rotary flash evaporator. A mixture of surfactant and cholesterol should be prepared and introduced into round bottom flask on rotary evaporator by sequential spraying of aliquot sonto carrier's surface. The evaporator has to be evacuated and rotating flask can be rotated in water bath under vacuum at 65-70 ^{o} C for 15-20 min. This process has to be prepared

until all of the surfactant solution had been applied. The evaporation should be continued until the powder becomes completely dry.

Advantages

It's a simple method suitable for hydrophobic drug without concerns of instability or susceptibility of active pharmaceutical ingredient to hydrolysis.

Disadvantages

1. Method is time consuming and involves specialized equipment with vacuum.
2. The thin film approach allows only for a predetermined lot sizes so material often wasted so minute quantities or small dose batch can be tedious one.

FACTORS AFFECTING ENTRAPMENT EFFICIENCY AND SIZE OF VESICLES [2,11]

Entrapment efficiency is the measure of solute retention. High entrapment efficiency means a less time and less effort needed to remove the unentrapped drug. Entrapment efficiency and vesicular size are important parameters to predict the stability of the dispersion. If the prepared formulation remains the unchanged vesicular size and entrapment efficiency even after storage, hence the formulation considered stable.

NON-IONIC SURFACTANTS

The non-ionic surfactant used act as vesicle forming agent, the amount and its nature will affect the entrapment and vesicular size.

A. Nature of non-ionic surfactant

The entrapment efficiency and vesicular size depends upon the HLB value, chemical nature and phase transition temperature. The surfactant with high or low HLB value has high entrapment efficiency with some approaching 100%. In addition to HLB, the chemical structure of the surfactant i.e., the alkyl chain length will also affect the entrapment, which is directly proportional to the phase transition temperature. The higher the alkyl chain higher will be the entrapment efficiency; as it is having high phase transition temperature thereby it is more likely to form orderly

gel form and hence less leaky. While surfactant with lower phase transition temperature are likely to form less orderly liquid. The increase in leakiness means it has less entrapment efficiency. This is evident from the report that, Sorbitan monooleate (span 20, span 80) and Stearate Sucrose Ester shows higher entrapment efficiency, as their alkyl chain is longer thereby higher phase transition temperature. Whereas, Tween 20 and 80 have higher HLB, value and hence showed lower entrapment compared to span series. While Tween 80 has comparatively higher efficiency than Tween 20, because of higher phase transition temperature due to longer alkyl chain.

B. Amount of surfactant

The increase in surfactant amount will increase the entrapment efficiency because the surfactant being the vesicle forming agent.

Table 3: Physical Properties of Non-ionic surfactants

S.No.	Surfactant	Synonyms	Properties
1.	Sorbitan Monolaurate	Span 20, Sorbitan Monodecanoate	Tc : 16ºC Density: 1.032 g/mL at 25°C (lit.) Flash point:>230°F HLB value: 8.6
2.	Sorbitan Monopalmitate	Span 40	Tc : 42ºC Flash point:113ºC Melting point: 46-47ºC HLB value: 6.7
3.	Sorbitan monostearate	Span 60, Sorbitan monooctadecanoate	Tc : 53ºC Flash point: >110ºC Melting point: 54-57ºC HLB value: 4.7

4.	Sorbitan Monooleate	Span 80, Sorbitan (Z)-mono-9-octadecenoate	Tc : -12ºC Flash point: >110ºC Density: 0.986 HLB value:4.3
5.	Polyoxyethylene (20)sorbitan Monolaurate	Tween 20	Density: 1.106 Aq.solubility: 100 g/L Boiling point: 100 ºC HLB value: 16.7
6.	Polyoxyethylene (20)sorbitan monopalmitate	Tween 40	Density: 1.05 Aq.solubility: 100 g/L Boiling point: 0.1 ºC HLB value: 15.6
7.	Polyoxyethylene (20)sorbitan Monostearate	Tween 60	Density: 1.081 Aq.solubility: 100 g/L HLB value: 14.9
8.	Polyoxyethylene (20)sorbitan Monooleate	Tween 80	Density: 1.064 Aq.solubility: 5-10g/100 mL at 23 ºC Flash point: >110 ºC HLB value: 15.0

CHOLESTEROL

Cholesterol that acts as cementing agent, when its concentration is increased which will considerably decrease the entrapment efficiency. It will retard the afflux profile of entrapped drug. The reason behind decrease in the entrapment efficiency due to increase in cholesterol is that with higher amounts of cholesterol may compete with the drug for packing space within the bilayer, hence excluding the drug as the

amphiphiles assemble into the vesicles. However, the effect of cholesterol will vary the entrapment efficiency according to the surfactant used. In span series the entrapment efficiency was increased with cholesterol to some extent, further increase lead to the decreased entrapment efficiency. This is explained to be due to the following fact that a small increase in cholesterol increases bilayer hydrophobicity and stability, thus the permeability is decreased, as it efficiently traps the drug in the bilayer as vesicles are formed. However, the cholesterol may compete with the drug as the concentration is beyond certain limit.

CHARACTERIZATION OF PRONIOSOMES[17,19]

Table 4: Shows methods for the characterization of proniosomes

Parameter	Method/ instrument
Particle size & size distribution	Lazer diffraction particle size analyzer, Photon correlation spectroscopy(PCS)
Vesicle size	Lazer diffraction particle size analyzer
Shape & surface morphology	Scanning electron microscopy(SEM) Transmission electron microscopy(TEM) Optical microscopy
Angle of repose	Funnel method
Sieve fractionation	Fritsch analysette sieve shaker
Aerodynamic behavior	Twin-Stage impinger
Spontaneity(Rate of hydration)	Neubaur's chamber
Separation of unentrapped drug	Centrifugation Cellophane dialysis tubing
Determination of entrapment efficiency	Vesicle lysis using alcohol and propylene glycol Dialysis method
In vitro drug release studies	Franz diffusion cells Keshary-chein diffusion cell Cellophane dialyzing membrane USP dissolution apparatus-I Spectarpor molecular porous membrane tubing *In vitro* skin permeation studies

Provesicles are characterized for vesicle size and morphology, entrapment efficiency, In vitro release and permeation studies, In vivo studies, stability studies etc.

Vesicle size and morphology -Vesicle morphology

It involves the measurement of size and shape. The size of the vesicles can be measured by light scattering method and optical microscopy in two conditions i.e: with agitation and without agitation. Hydration without agitation results in largest vesicle size. Surface morphology means roundness, smoothness and formation of aggregation; it can be studied by scanning electron microscopy and transmission electron microscopy.

Optical microscopy

In optical microscopy, small amounts of the formed niosomes are spread on a glass slide and examined for the vesicles structure and the presence of insoluble drug crystals using ordinary light microscope with varied magnification power 100x.

Scanning electron microscopy

Particle size of proniosomes is very important characteristic. The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of proniosomes were studied by Scanning Electron Microscopy (SEM). For scanning electron microscopy, the niosomes are mounted on an aluminum stub using double sided adhesive carbon tape. Then the vesicles are sputter coated with gold palladium (Au/Pd) using a vaccum evaporator and examined using a Scanning electron microscopy equipped with a digital camera at 25kV accelerating voltage.

Transmission electron microscopy

The morphology of hydrated niosome dispersion prepared from proniosome was also determined using transmission electron microscopy (TEM). The noisome dispersion is applied to a carboncoated 300 mesh copper grid and left to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion is removed by absorbing the drop with the corner of a piece of filter paper. Then drop of aqueous solution of uranyl acetate is applied. The remaining solution is removed by absorbing the liquid with the tip of a piece of filter paper and the sample is air dried and observed under transmission electron microscope.

Entrapment Efficiency

Various methods can be used to evaluate the loading capacity of proniosomal systems such as dialysis method, gel filtration and centrifugation method. In dialysis method, amount of entrapped drug can be obtained by subtracting the amount of unentrapped drug from total drug incorporated.

Entrapment efficiency(EE) = Amount of drug entrapped/total amount of drug×100

***In -vitro* release and permeation studies**

In- vitro release and skin permeation studies for proniosomes were determined by different techniques like franz diffusion cell, dialysis tubing and reverse dialysis. In case of dialysis, the prewashed dialysis tubing is used which can be hermatically sealed, the proniosomes are placed in it and then dialysed against a suitable dissolution medium at a room temperature. The samples are withdrawn from the medium at suitable interval, centrifuged and analysed spectrophotometrically (UV, HPLC).

Stability studies

Stability studies are carried out by storing the proniosomes at various temperature conditions like refrigeration, room temperature and elevated temperature according to ICH guidelines. Drug content and variation in the average vesicles diameter is periodically monitored. According to ICH guidelines the stability studies for dry proniosomes powder should be studied for accelerated stability at 75% relative humidity as per international climate zones and climate conditions.

CLINICAL APPLICATIONS OF PRONIOSOMES[17,19]

Approaches to stabilize niosomal drug delivery system without affecting its properties of merits have resulted in the development of the promising drug carrier, proniosomes. Proniosomes is dry formulation using suitable carrier coated with non ionic surfactants and can be converted into niosomes immediately before use by hydration. These proniosome-derived niosomes are as good as or even better than conventional niosomes. The application of proniosomal technology is widely varied and can be used to treat a number of diseases. The following are the few uses of proniosomes which are either proven or under research.

1. Anti-neoplastic Treatment :

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism; prolong circulation and half life of the drug, thus decreasing the side effects of the drugs. Niosomal entrapment of Doxorubicin and Methotrexate (in two separate studies) showed beneficial effects over the unentrapped drugs, such as decreased rate of proliferation of the tumor and higher plasma levels accompanied by slower elimination.

2. Leishmaniasis :

Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of pronoisome in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment.

3.Delivery of Peptide Drugs :

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated. In an *in-vitro* study conducted by Yoshida et al, oral delivery of a vasopressin derivative

entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide.

4. Uses in Studying Immune Response:

Proniosomes are used in studying immune response due to their immunological selectivity, low toxicity and greater stability. Niosomes are being used to study the nature of the immune response provoked by antigens.

5. Proniosomes as Carriers for Haemoglobin :

Niosomes can be used as carriers for haemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for hemoglobin in anemic patients.

6. Proniosomes used in Cardiac Disorders:

Proniosomal carrier system for captopril for the treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a transdermal drug delivery system for captopril was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acidesters, cholesterol, lecithin prepared by coacervation-phase separation method. The formulated systems were characterized *in-vitro* for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for 4 weeks. The current study was to investigate the feasibility of proniosomes as transdermal drug delivery system for losartan potassium.

7. Sustained Release:

The role of liver as a depot for methotrexate after niosomes are taken up by the liver cells. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation.

8. Localized Drug Action:

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time

reduces its systemictoxic effects e.g. Antimonials encapsulated within niosome are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity. The evaluation of niosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and antileishmanial therapy.

9. Hormonal Therapy:

A proniosome based transdermal drug delivery system of levonorgestrel (LN) was developed and extensively characterized both *in-vitro* and *in-vivo*. The proniosomal structure was liquid crystalline compact niosomes hybrid which could be converted into niosomes upon hydration. The system was evaluated *in-vitro* for drug loading, rate of hydration (spontaneity), vesicle size, polydispersity, entrapment efficiency and drug diffusion across rat skin. The effect of composition of formulation, amount of drug, type of Spans, alcohols and sonication time on transdermal permeation profile was observed. The stability studies were performed at 4°C and at room temperature. The biological assay for progestational activity included endometrial assay and inhibition with the formation of corpora lutea. The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraception.

10. Cosmetics formulation:

Large number of cosmetic preparations available in the market is utilizing niosomes and liposomes as a carrier for delivery of actives. Liposomes were prepared using unacceptable organic solvents, whose traces in the final preparation can cause harm to the skin. It is proved that proniosomes are as effective as noisome and liposomes, but their preparation, handling, storage and transportation make them superior over others. The therapeutic agents which can be utilized for incorporation into proniosomal carrier systems include, moisturizing, nutritional, anti wrinkle, anti-ageing, cleansing, sunscreen particles, etc.

11. NSAID Application:

Ketorolac trimethamine (KT) is a nonsteroidal agent with potent analgesic and moderate anti-inflammatory activity. The drug is currently administered intramuscularly and orally in divided multiple doses for short-term management of postoperative pain (30 mg q.i.d. by IM injection and 10 mg q.i.d. as oral tablets). This frequent dosing, which results in unacceptable patient compliance, is required due to the short half-life of the drug (4–6 h). Therefore, an alternative noninvasive mode of delivery of the drug is needed. Transdermal delivery certainly appears to be an attractive route of administration to maintain the drug blood levels of KT for an extended period of time . Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), are used in the treatment of dysmenorrheal various acute and chronic musculoskeletal disorders like rheumatoid arthritis, osteoarthritis etc., and also as potent analgesics . However, the use of piroxicam has been associated with a number of gastrointestinal disorders. Dermal delivery is an alternative route, butrequires a formulation which ensures the deep skin penetration. Aceclofenac In the present study the slurry method was used for the preparation and optimization study of aceclofenac, as this method is simple and easy to scale up. Aceclofenac is a poorly water soluble, non-steroidal anti-inflammatory drug which acts specifically on inflammatory sites and thereby decreases the inflammation. It is highly effective as an anti-inflammatory drug for various inflammatory conditions like rheumatoid arthritis, osteoarthritisand ankylosing spondylitis.

2 REVIEW LITRATURE

1. **Amit kumar *et al*.**[27] developed maltodextrin based stavudine proniosomes drug delivery systems were developed by using nonionic surfactant:cholesterol at different concentrations. All the prepared formulations are subjected into physicochemical evaluations. These results the entrapment efficiency of niosomes prepared from proniosomes at varied concentration of surfactants:cholesterol keeping stavudine concentration constant. The percentage entrapment efficiency range of 56.22 ± 0.15 to 94.45 ±0.16.Higher surfactant concentration viz ,Span 60, Span 40 shows higher entrapment efficiency which might be due to the high fluidity of the vesicles. The larger vesicle size may also contribute to the higher entrapment efficiency.

2. **Mohamed Nasr *et al*.**[28] developed Celecoxib proniosomes and evaluate the influence of proniosomal formulation on the oral bioavailability of the drug in human volunteers. Proniosomes were prepared by sequential spraying method .which consisted of cholesterol, Span 60 and dicetyl phosphate in amolar ratio of 1:1:0.1.The average entrapment percent of Celecoxib proniosomes derived niosomes was about 95%.The prepared proniosomes showed marked enhancement in the dissolution of Celecoxib as compared to pure drug powder. The bioavailability of single dose of Celecoxib proniosomal formulation and a conventional marketed capsule was studied in human volunteers. These results show that the proniosomal formulation significantly improved the extend of Celecoxib absorption than the conventional capsule.

3. **D.Akilesh *et al*.**[29] developed and optimized of maltodextrin and mannitol and Sorbitol based proniosomes were prepared by slurry method with different surfactant to cholesterol ratio. All these formulations are subjected into physicochemical evaluations. The formulation based maltodextrin showed higher entrapment efficiency of 82.64 ± 1.25 and *in-vitro* release of of 98**%** at the end of 24 hr was found to be best among the various formulations.

4. **Preethy Cheriyan *et.al*.**[30] developed maltodextrin based proniosomes of Cefuroxime axetil. These formulations are prepared by varying the surfactant – lipid loading in each formulation. All these formulations are subjected into physicochemical evaluations. From the results of entrapment studies, it could be concluded that the formulation PN4 which is having surfactant:lipid concentration as 1:1 was the best formulation. This study suggests that the formulation can provide consistent and prolonged release of Cefuroxime axetil from the different niosomal formulations. It will lead to sustained action of the entrapped drug associated with frequent administration.

5. **T.Sudhamani *et al*.**[71] developed Ibuprofen loaded maltodextrin based proniosome were prepared by slurry method with different surfactant to carrier ratio. All these formulations are subjected into physicochemical evaluations. These results the formulation F4 which showed higher entrapment efficiency of 96.57 ± 1.08 % and *in-vitro* cumulative drug release of 92.16% at the end of 12 hr was best formulation.

6. **Akhilesh Dubey *et al*.**[72] developed Lornoxicam loaded maltodextrin based proniosomes were prepared by slurry method with different surfactant to cholesterol ratio. All these formulations are subjected into physicochemical evaluations. These results the formulation F3 which showed higher entrapment efficiency of 72.69% and *in-vitro* release of 91.17% at the end of 24 hr,was found to be best formulation among the 7 formulation. Higher entrapment efficiency of the vesicles span 60 is predictable because of its higher alkyl chain length. The proniosomal formulations have the higher entrapment efficiency which might due to the high fluidity of the vesicles. Very low cholesterol content was found to cause low entrapment efficiency ,which might be because of the vesicles.

7. **Tamizharasi Sengodan *et al*.**[33]developed Indomethacin loaded maltodexrin based proniosome by slurry method with different surfactant to cholesterol ratio. Preparation of proniosomes was optimized for highest percentage drug entrapment. All these formulations are subjected into physicochemical evaluations. Formulation F4 showed highest entrapment efficiency of 81.28 ± 4.38%w/w. The proniosomal formulations having low cholesterol content was found to cause low entrapment efficiency which

might be because of leakage of the vesicles. The higher entrapment may be explained by cholesterol content. These are reports that entrapment efficiency increase with increasing cholesterol content and by the usage of span 60 which has higher transition temperature.

8. **Sharda sambhakar *et al* .**[34] developed dry free-flowing proniosomes Cefuroxime axetil has been prepared with sorbitol by spray coating method. Span 40, 60 and 80 have been used in different molar ratio with cholesterol and stearylamine. Proniosomes are characterized by physicochemical evaluation and *ex-vivo* permeation study. Entrapment efficiency of span 60 was found to be maximum. Both proniosomes and niosomes indicate the similar controlled release profile.

9. **Meenakshi K. Chauhan *et al*.**[35] developed maltodextrin based proniosomes to improve the oral delivery of Ramipril by using slurry method. All these formulations are subjected into physicochemical evaluations. The release rate of formulation compared to marketed formulation that the formulation had high encapsulation efficiency and provided a sustained release over 24 hr.

10. **Prakash S Goundanavar *et al*.**[36] developed proniosomes of Irinotecan hydrochloride trihydrate were prepared by slurry method using different surfactants, cholesterol and dicetyl phosphate. All these formulations are subjected into physicochemical evaluations *in-vivo* targeting. These results proniosomes offer a suitable alternative colloidal carrier approach to achieving drug targeting. Proniosomes containing Irinotecan are retained at targeted sites and are capable of releasing drug for an extended period of time.

11. **R.Parthibarajan *et al.***[77] developed methotrexane entraped Proniosomes were prepared by slurry method using cholesterol, surfactant(span80), maltodextrin. Preparation of proniosomes was optimized for highest percentage drug entrapment. Increasing the span 80 concentration might increase the drug entrapment efficiency. Proniosomes could be used as a drug carrier for producing prolonged activity.

12.**D.Akhilesh *et al*.**[78] developed Gliclazide loaded maltodextrin based proniosomes. The results reported here indicate that proniosomes are very promising as drug carriers. The present formulation study on Gliclazide is an attempt to prepare proniosome based niosomal drug delivery system using maltodextrin as carrier and to evaluate its performance. The proniosomes with various types and contents of nonionic surfactant and cholesterol is evaluated in this study. An ideal or best formulation of proniosomes based niosome is said to be one which gives high entrapment efficiency with desirable sustained release. In this study entrapment efficiency is found to be cholesterol:surfactant ratio dependent. The release rate also found to be dependent of cholesterol: surfactant ratio for effective proniosomes.

13. **Ajay solanki *et al*.**[39] prepared, characteraized, optimization and carried out stability studies of Aceclofenac proniosomes. This investigation was to prepare, characterize and optimize the Aceclofenac proniosomes using central composite design and carry out stability studies. Three independent variables selected were molar ratio of drug to lipid (X1), surfactant loading (X2) and volume of hydration (X3). Based on central composite design, 16 batches of proniosomes were prepared by slurry method and evaluated for the percentage drug entrapment (PDE) and mean volume diameter (MVD). The PDE and MVD (dependent variables) and the transformed values of independent variables were subjected to multiple regressions to establish a second order polynomial equation. Contour plots were constructed to further elucidate the relationship between the independent and dependent variables.

14. **Lakshmi G. et al**[40]. designed and characterized of Vinblastine sulphate loaded proniosome for cancer therapy . This investigation was to prepare, characterize and optimize the Vinblastine sulphate loaded proniosomes for overall improvement in the physical stability and to prolong the release time in a controlled manner there by increasing its efficacy and to reduce its toxicity and to study the suitability of proniosomes as the carrier of drug. Proniosomes of Vinblastine sulphate were prepared by slurry method. The results obtained for the present study clearly revealed that proniosomes containing Vinblastine sulphate are capable of releasing their drug for the extended period of time there by increasing the efficacy of drug.

15.**B.Agaiah Goud *et al*.**[41] formulated and evaluated of Megesterol proniosomal systems. The various proniosomal systems developed were span 20:megesterol (50:50%), Span20:Megesterol(70:30%), Span20 50% and 50% Megesterol+cholesterol (50:50), and Span 20, 70% and 30% Megesterol+cholesterol (50:50). Out of all the compositions the one with 70% span 20 and 30% Megesterol:cholesterol (50:50) exhibited slower release.

16. **Rishu Kakkar *et al*.**[42] developed non-ionic surfactant vesicles of Valsartan, an angiotensin II inhibitor, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and in-vitro drug release. The encapsulation efficiency of proniosomes prepared withSpan60 was superior to that prepared with Span 40. A preparation with 9:2:9 ratio of Span 60,cholesterol and lecithin gave maximum encapsulation efficiency (71.50%) and release results (Q24h=75%) as compared to other compositions.

17. **Hemant N.Patil *et al.*[44]** carried out formulation development and evaluation of proniosomal gel of Carvedilol .To optimize the formulation, various proniosome gels composed of various ratios of sorbitan fatty acid esters, polysorbates, cholesterol, lecithin were prepared by coacervation-phase separation method. Proniosomal gel (PNG) formulations of Carvedilol were characterized for vesicular shape & size, entrapment efficiency, permeation study, stability study, pH and viscosity of gel. The effects of cholesterol, lecithin and different non-ionic surfactants on transdermal permeability profile of Carvedilol were also assessed. The percent encapsulation of Carvedilol in proniosome with Tween surfactants revealed a very high entrapment efficiency than the span surfactants.

18. **Madhu B.K *etal*.**[45] developed proniosomal formulation of Diclofenac Sodium by slurry method based on non-ionic surfactants (Tween 60), cholesterol and maltodextrin as a carrier. Four formulations were prepared using maltodextrin as a carrier, cholesterol, Tween 60, carbopol 934P as polymers. Proniosomal formulations were prepared using slurry method. The proniosomal formulation was evaluated for FT-IR study, scanning electronmicroscopy, stability study, In-vitro release study respectively. The formulation F4 which showed higher entrapment efficiency of 77.23% with Tween 60respectively and in-vitro releases of 77.01% at the end of 24

hrs was found to be best among the all 4 formulations. From these results proniosomal formulation could be a promising deliverysystem for Diclofenac Sodium with improved bioavailability and better controlled drug release.

19. **Sandeep loona *et al*.**[46] developed proniosomal carrier system of Metformin hydrochloride for the treatment of type – 2diabetes mellitus that is capable of delivering entrapped drug over an extended period of time. Proniosomes of metformin hydrochloride were prepared by coacervation phase separation method. The potential of proniosomes as a transdermal drug delivery system was estimated by encapsulating the drug in various formulations of proniosomal gel composed of different ratios of Span 60/Span 40, cholesterol and lecithin. The prepared systems were characterized for encapsulation efficiency, size, zeta potential analysis, in-vitro drug release and vesicular stability at different storage conditions. The results showed that the encapsulation efficiency of proniosomes prepared with Span 60 was superior to that prepared with Span 40. A formulation (i.e. PNG2) with 9:2:9 ratio of span 60, cholesterol andlecithin gave maximum encapsulation efficiency (76.8 %), good zeta potential (-51.9) and lowest drug release percent after 24 hrs(75.9%). It is evident from the study that the Metformin proniosomal gel is promising prolonged drug delivery system and has reasonably good stability characteristics.

20. **Hayder K. Abbas *et al*.**[47] prepared Metoprolol tartrate niosomal gel as transdermal drug delivery system and also to evaluate procedure related variables like type of surfactant and release of drug from niosomes. Metoprolol tartrate niosomes are formulated by coacervation-phase separation method using different types of non-ionic surfactant, lecithin and cholesterol. The prepared formulations are estimated for its entrapment efficiency, vesicle size, the compatibility of the drug and additives used and morphological characters .Higher entrapment efficiencies are obtained with Span 40 and span 60 (86.6%±8.07 and 78.09±15.44 respectively) and the release rate at 11 hr from span 40 niosomes is found to be 31.18%. In conclusion, the niosomal gel formulation could be a promising transdermal delivery system for Metoprolol tartarate with prolonged drug release profiles.

21. **Anindita D *et al.*[48]**formulated and evaluated Tretinoin proniosomal gel and to carry out comparative skin irritation study with conventional Tretinoin solution and Tretinoin conventional gel. The proniosomal dispersion was prepared using different grades of non-ionic surfactants and cholesterol in different ratios along with Tretinoin. The proniosome vesicles prepared with Span 60, 40 and cholesterol in formulation PN9 showed maximum entrapment efficiency(76.77±1.54) .The prepared proniosome vesicles were incorporated into Carbopol gel (1%) base to prepare Tretinoin proniosomal gel. The *In-vitro* diffusion study carried out using sigma dialysis membrane showed sustained release pattern of Tretinoin from proniosomal gel formulation.

22. **Peeyush Vasista *et al*.[49]** developed a proniosomal carrier system for Lisinopril and Hydrochlorothiazide (HCTZ) drug combination for prophylaxis and treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a transdermal drug delivery system for Lisinopril and hydrochlorothiazide was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, drug determined by factorial design and prepared by coacervation-phase separation method. The formulated systems were characterized *in-vitro* for size, drug entrapment, drug release profiles and vesicular stability at different storage conditions. The optimized formulations of HCTZ and Lisinopril were obtained by employing factorial design at two phases. The method of proniosome loading resulted in an encapsulation yield of 37.01± 1.26% and 59.94± 1.56% for HCTZ and Lisinopril respectively. *In -vitro* studies of HCTZ and Lisinopril proniosome was carried by drug diffusion through cellophane membrane and *Ex-vivo* skin permeation studies, the percent drug released after 24 hrs was found ranging in 79-66 % and 48-61% of total drug entrapped for Lisinopril and HCTZ respectively. From these result of this study that proniosomes are a promising prolonged delivery system for HCTZ and Lisinopril .

23. **Alpana Ram *et al.*[50]**developed proniosomal carrier system for Hydralazine for prophylaxis and treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a

transdermal drug delivery system for Hydralazine was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, lecithin prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Vesicle size and drug permeation was greater for formulation containing Span 40, 60 due to its high hydrophobicity which resulted in smaller size of vesicle. Thus proniosomal gel will be suitable drug delivery system for Hydralazine HCl due to ease of preparation and incorporation of less no.of excipients.

24. **M.Intakhab Alam *et al*.**[51] worked on pharmacodynamic evaluation of proniosoma transdermal therapeutic gel containing Celecoxib. All the prepared formulations were subjected to physicochemical evaluations and anti-inflammatory studies. The entrapment was> 90%. The selected proniosomal gel (N1LE3)produced 100% inhibition of paw oedema in rats up to 8 h after carrageenan injection. It produced 95% and 92% inhibition after 12 h and 24 h, respectively. These results indicate that proniosomes are a promising carrier for the transdermal delivery of Celecoxib.

25. **Thulasi Chowadry *et al*.**[52] studied *in-vitro* dynamics of ibuprofen incorporated proniosomal gel. Different proniosomal gels of ibuprofen were formulated with Span 20/Span 80 and soya lecithin using the method described in literature. In all formulations cholesterol concentration was kept constant. Proniosomal gel prepared using Span 80 showed higher flux across the membrane due to its leaky membrane. The order of ibuprofen release from the proniosomal gel was PN2>PN4>PN1>PN3.The optimized proniosomal gel formulation PN3 containing Span 20 exhibited prolonged ibuprofen release profiles. Fickian diffusion mechanism was observed with the PN3 formulation which was due to the sustained release property. The results indicated that the proniosomal gel would be an effective transdermal delivery system for ibuprofen.

26. **Kapil Kumar *et al*.**[53]developed proniosomal carrier system of Curcumin for transdermal delivery. Proniosomes of Curcumin were prepared by encapsulation of the drug in a mixture of Span80, cholesterol and diethyl ether by ether injection

method, and then investigated as a transdermal drug delivery system (TDDS). The formulated systems were characterized for size, drug entrapment, angle of repose, hydration rate and vesicular stability under various storage conditions. *In-vitro* release studies were performed using albino rat skin. The method used for preparing proniosome resulted in an encapsulation yield of 82.3 – 86.8%.One of the formulations (PG1) showed prolonged in vitro drug release of 61.8% over a period of 24 h. It is evident from this study that proniosomes are very stable and promising prolonged delivery system for Curcumin.

27. **Dr. A.Seetha Devi *et al.*[54]**developed Candesartan cilexetil proniosomal gel by coacervation phase separation method by using different surfactants, cholesterol and soya lecithin in 9:1:9 and 9:2:9 ratios. The prepared proniosomal gel formulations were evaluated for vesicle size analysis, surface morphological studies, encapsulation efficiency, *In-vitro* drug release, *ex-vivo* skin permeation studies and vesicular stability at different storage conditions. Encapsulation efficiency of proniosomes formed from Span 60, Span 40, Span20, and Span 80 was found high compared with proniosomes prepared from Tweens (Tween 20 and Tween 80). An optimised preparation with 9:2:9 ratio of Span 60, cholesterol and lecithin gave maximum encapsulation efficiency (92.29%) and showed drug release (95.89±0.26%) in a controlled manner with a flux value of 1.89 µg/cm2 /hr and permeability co efficient value of 0.094 cm2/hr as compared to other compositions. It is evident from this study that proniosomes are a promising prolonged delivery system for Candesartan cilexetil and have reasonably good stability characteristics.

28. **Sandhya P *et al*.[55]** developed proniosomes as a transdermal drug delivery system for Losartan potassium was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of Span (sorbitan esters) and Tween (polyoxythylene sorbitan esters), cholesterol, lecithin prepared by coacervation-phase separation technique. The formulated systems were characterized for Vesicle physical analysis, drug entrapment, rate of spontaneity, FTIR Studies, *in-vitro* release kinetics, drug release profiles, and vesicular stability at different storage conditions for Losartan potassium proniosomal gel were carried out according to ICH Guidelines. The results showed that the type of surfactant incorporated altered

the entrapment efficiency of proniosomal gel and higher entrapment efficiency of 98.24% was obtained with the proniosomal gel PNG F4.

29. **Varsha gadekar *et al*.**[56]developed non-ionic surfactant based Proniosome Gels of Naproxen sodium, an cox II inhibitor, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and *in-vitro* drug release. Stability study was carried out to investigate the leaching of drug from the proniosomal system during storage. The results showed that Naproxen in all the formulations was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of Naproxen from proniosomes were observed upon varying the type of surfactant and cholesterol content. The encapsulation efficiency of proniosomes prepared with Span 40:60 was superior to that prepared with all Span prepration . A preparation with Span 40: 60, cholesterol and lecithin gave maximum encapsulation efficiency (84.61%) and release results (Q24h= 81%) as compared to other compositions.

30. **Rajesh Asija *et al*.**[57]developed non-ionic surfactant vesicles of Acyclovir, an antiviral, were prepared by coacervation phase separation method. The prepared systems were characterised for encapsulation efficiency, shape, size and *in-vitro* drug release scanning electron microscopy (SEM). Proniosomal prepared by using different polymers Span 40, Span 60, Span 20, Tween 20, Tween 80 and cholesterol and soya lecithin.The encapsulation efficiency of proniosomes prepared with Span 60,cholesterol and lecithin gave maximum encapsulation efficiency (89.70%) as compared to other compositions.The niosomal gel formulation could be a promising transdermal delivery system Acyclovir with prolonged drug release profiles.

31. **Sundarapandian Ramkanth *et al*.**[59] prepared proniosome gel type transdermal delivery system of Atenolol was prepared by coacervation phase separation method. The prepared formulations were evaluated for vesicle size, entrapment efficiency, *in-vitro* drug loading, and drug release studies. The release of drug had shown considerable improvement in controlled manner from the prepared gel formulation. It was observed that Span 40 & 60 (A 8) based formulations shows vesicles of minimum size and higher entrapment efficiency compared to the other formulations. Proniosomal transdermal therapeutic system (A 8) was found to be the optimized

formulation as it posses good drug release and shows permeation in a steady-state manner over a desired period of time.

32**. Aliya Parveen *etal*.**[60] developed muco adhesive microspheres of Lamivudine. Microspheres were formulated using sodium alginate (5%) with mucoadhesive polymer (Chitosan 1%) and copolymer Sodium CMC HPMC, Xanthan gum (XG) in concentration of 1% (Chitosan1% + HPMC1%) (1%) retarding agents and 10% of Calcium chloride(CaCl2), Aluminum sulphate (AlSO4) as cross linking agents by employing Ionic Gelation Technique. Amongthe prepared microspheres (F8) formulation in which AlSO4 was used as cross linking agent, portray better sustained release for more than 12hrs.

33. **K.Prakash *etal*.**[61] developed microcapsules for the controlled release of Lamivudine using various cellulose polymers by the solvent evaporation method. The prepared microcapsules were characterized for the percent drug content, entrapment efficiency, FTIR, DSC, scanning electron microscopy (SEM) and *in-vitro* dissolution studies. Microcapsules were spherical and free flowing. The entrapment efficiency was 76-86%.The release of drug from the microcapsules extended up to 8 to 12 hours. FTIR and DSC thermograms showed the stable character of Lamivudine in the microcapsules.

34. **R.Ruben Singh**[62] .developed oral sustained release tablets of Lamivudine Hydrochloride using different natural polymers such as Chitosan, Guar gum and Xanthan gum. The Lamivudine Hydrochloride oral sustained release tablets were prepared by using wet granulation method. The formulated different ratio of oral sustained release tablets of Lamivudine were evaluated by different parameters. The prepared granules were evaluated for angle of repose, bulk density, tapped density, compressibility index and hausner's ratio. The tablets were evaluated to thickness, weight variation test, hardness, friability, drug content, *in-vitro* release and kinetic release studies. The results conclude that FL-7 can be considered as a optimized formula for sustained release of drug for 24 hours.

35.**Varun Dasari *et al*.**[63] formulated and carried out *in-vitro* evaluation of Lamivudine multiunit floating dosage forms using novel lipoidal polymers. In the

present study, Lamivudine was selected as model drug in the design as GFDDS using various lipoidal/ fatty polymers. Lamivudine complies with all the requirements that are suitable for a drug candidate to be formulated as GFDDS, as it has specific site of absorption in upper part of GIT. Since the halflife of Lamivudine is around 4Hours, multiple doses are needed to maintain plasma concentration for a good therapeutic response and improved patient compliance. Prescriptional 100 mg dose of Lamivudine wass elected to sustain the release in order to maintain its effective plasma drug concentrations for 12 hours.

36. **Harekrishna Roy *et al.*[64]** developed sustained release dosage form using various grades of hydrophilic polymers, Hypromellose (hydroxyl-propyl methylcellulose HPMC K4M, HPMC K15M and HPMC K100M) and Povidone K30 as binder solution in a matrix-controlled drug delivery system of Lamivudine. Six tablet formulations were prepared by wet granulation technique. The results obtained revealed that HPMC K4M at a concentration of 25% in formulation (F2) was able to sustain the drug release for 24 h and followed the Higuchi pattern.

37. **Parvin Patil *et al.*[65]** developed solid lipid nanoparticles of Lamivudine for brain targeting .Solid lipid nanoparticles prepared by using emulsion solvent diffusion technique. The formulation were characterized for surface morphology, size, percentage drug entrapment and drug release. The optimum rotation speed, resulting into better drug entrapment and percentage yield, was in the range of 1000-1250 r/min. The in-vitro cumulative % release from optimized formulation was found to 40-50% in PBS and SGF for 10 hour.

38. **A.Abdhul Hasan Sathali *et al.*[66]**formulated and evaluated fast dissolving tablets of Lamivudine to prevent mother to child transmission (MTCT) of HIV virus in perinatal infants. The tablets were prepared by direct compression method, using various superdisintegrants like sodium starch glycolate, croscarmallose sodium, and crospovidone at various concentrations(2%-10%). The results of precompression studies reveals that the powder blends of all formulations acquire good flow properties. From the results of post compression studies for tablets of all formulations, it was concluded that the formulation containing 10% crospovidone as

superdisintegrants emerged as overall best formulation with lowest disintegration time and highest drug release rate.

39. **Ashish Dev *et al.*[67]** prepared of poly(lactic acid)/chitosan nanoparticles for anti-HIV drug delivery Poly(lactic acid) (PLA)/chitosan (CS) nanoparticles were prepared by emulsion method for anti-HIV drug delivery applications. The hydrophilic antiretroviral drug Lamivudine was loaded into PLA/CS nanoparticles. The *in-vitro* drug release studies showed that drug release rate was lower in the acidic pH when compared to alkaline pH. Drug release rate was found to be higher in the 6% drug loaded formulation when compared to 3% drug loaded formulation. These results indicated that the PLA/CS nanoparticles are a promising carrier system for controlled delivery of anti-HIV drugs.

40. **Subheet Jain *et al.*[68]** developed ethosomes for transdermal delivery of Lamivudine. The optimized ethosomal formulation showed 25 times higher transdermalflux (68.4 ± 3.5 μg/cm2/h) across the rat skin as compared with that of Lamivudine solution (2.8 ± 0.2 μg/cm2/h). Results of cellular uptake study showed significantly higher intracellular uptake of ethosomes (85.7% ± 4.5%) as compared with drug solution (24.9% ± 1.9%). The results of the characterization studies indicate that lipid perturbation along with elasticity of ethosomes vesicles seems to be the main contributor for improved skin permeation.

3. AIM AND PLAN OF WORK

AIM OF WORK

The aim of the present study is

- To formulate Lamivudine Proniosomal drug delivery system by two different methods using different non-ionic surfactants, cholesterol, maltodextrin and lecithin.
- To study the effect of varying surfactant concentration on entrapment efficiency, drug release, and mean vesicle size.
- To sustain the drug release of Lamivudine proniosomes.
- To provide a safe dosage form with lesser side effects.
- To reduce the frequency of administration thereby improving the patience complaince.

PLAN OF WORK

The present work is carried out to design and evaluate Lamivudine proniosomes. Conventional oral formulations of Lamivudine are administered multiple times a day because of its half – life (5-7 hrs). Treatment of AIDS using conventional formulations of Lamivudine are found to have a many drawbacks, such as accumulation of drug in multidose therapy and poor patient complaince. So the study is carried to overcome some of these problems by prolonging the rate of drug release.

PART-I

- Drug excipients compatibility studies – FTIR study.

PART-II

- Preparation of standard curve of Lamivudine .

PART-III

- Preparation of Lamivudine proniosomes by slurry method using different non-ionic surfactants (Span 40, Span 60, Tween 60) in different ratio. Concentration of Cholesterol , Maltodextrin were kept constant.

- ❖ Preparation of Lamivudine proniosomes by coacervation phase separation method using different non-ionic surfactants(Span 40, Span 60, Tween 60) in different ratio. Concentration of Cholesterol, Lecithin were kept constant.

PART – IV

- ❖ Evaluation of Lamivudine proniosomes
  - ✓ Mean Vesicle Diameter (MVD) by Binocular Microscopy.
  - ✓ Drug content
  - ✓ Entrapment efficiency by centrifugation method.
  - ✓ *In-vitro* release study of Proniosomal formulations and Lamivudine control in distilled water using dialysis membrane.

PART-V

- ✓ Comparison of Lamivudine proniosomes prepared by slurry method and coacervation phase separation method using entrapment efficiency and *in-vitro* drug release.
- ✓ To study the effect of surfactant concentration on entrapment efficiency, drug release, mean vesicle size.
- ✓ Optimization of best method from the methods (slurry method/ coacervation phase separation method).
- ✓ Optimization of best surfactant from slurry method using entrapment efficiency and *in-vitro* drug release.

PART – VI

- ❖ *In-vitro* release study and release kinetics of optimized formulation.

PART – VII

- ❖ Morphological studies of optimized formulation using Scanning Electron Microscopy.

PART – VII

- ❖ Stability studies of optimized formulation.

4. RATIONALE OF THE STUDY

RATIONALE OF THE WORK[32]

Although considerable progress has occurred in the development of the therapy for AIDS, the available antiretroviral drugs are not always effective, are frequently toxic and do not clear virus from the tissue reservoirs. The major aspect that makes HIV dangerous disease, is its ability to replicate itself. We could not find a cure so far due to the mutation process, which this virus undergoes resulting in the formation of several mutant groups. There are basically two strains of HIV virus : HIV1 and HIV2. HIV1 accounts for 95% of all infections worldwide.HIV2 is mainly seen in West African countries.

Antiretroviral(ARV) drugs are broadly classified by the phase of the retrovirus life –cycle that the drug inhibits. They are

- ❖ Nucleooside and Nucleotide reverse transcriptase inhibitors (NRTIs)
- ❖ Non- Nucleotide reverse transcriptase inhibitors (NNRTIs)
- ❖ Protease inhibitors
- ❖ Integrase inhibitors
- ❖ Maturation inhibitors

Nucleoside and Nucleotide reverse transcriptase inhibitors inhibit reverse transcription by being incorporated into the newly synthesized viral DNA strand as afaulty nucleotide. This causes a chemical reaction resulting in DNA chain termination. Non-Nucloside reverse transcriptase inhibitors inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function.

SELECTION OF FORMULATION[26]

Proniosomes is a surfactant coated vesicular drug delivery system. It is more advantageous over liposomes and niosomes because,

- Liposomes and niosomes are dispersed aqueous system and have a problem ofdegradation by hydrolysis or oxidation.
- Liposomes and niosomes require special storage and handling.
- Sedimentation, Aggregation or fusion on storage is usually seen.
- In liposomes, purity of natural phospholipids is also variable.

- Difficulty in sterilization, transportation, distribution, storage uniformity of dose and scale up.

RATIONALE FOR SELECTION OF THE DRUG

Lamivudine is one of the dideoxycytidine analogue Nucleoside Reverse Transcriptase inhibitor (NRTs) is the first nucleoside analogue to treat AIDS and chronic HBV infection. Lamivudine can be used in both cases of HIV (type 1 and type 2).

As there is no complete cure for the HIV, the patient has to take continuous medication to extend the lifespan but to continuous medication to the patient there occurs a lot of side effects and adverse effects of the high amount of dose administered daily with the conventional dosage form. In order to overcome the problem of frequent dosing of Lamivudine, the prolonged drug delivery system of proniosomes containing Lamivudine formulated.

SELECTION OF MATERIALS

The materials are selected in order to produce non-toxic, smaller homogenous discrete vesicles having high entrapment of drug.

Surfactant [24]

- The non-ionic surfactants (Sorbitan Monoesters) are selected for producing non-toxic drug delivery systems.
- The non-ionic surfactants are having permeation enhancement property.
- Among all the spans (Sorbitan Monoesters), the Span 60 Shows high entrapment ofdrug.

Cholesterol [24]

- Cholesterol is used as a membrane stabilizers.
- It resembles the biological membrane.
- Non-toxic.

Lecithin [24]

- Phosphatidyl choline is used as a membrane stabilizer.
- The egg lecithin is preferred over the soya lecithin because, the egg lecithin produce thesmaller vesicles.

Maltodextrin [22]

- It is a mixture of glucose, disaccharides and polysaccharides, obtained by the partial hydrolysis of starch.
- Maltodextrin is a flavorless, easily digested carbohydrate made from cornstarch. A maltodextrin is a short chain of molecularly linked dextrose (glucose) molecules, and is manufactured by regulating the hydrolysis of starch.
- A white or almost white, slightly hygroscopic powder or granules, freely soluble in water.

DISEASE PROFILE[7,31,32,38]

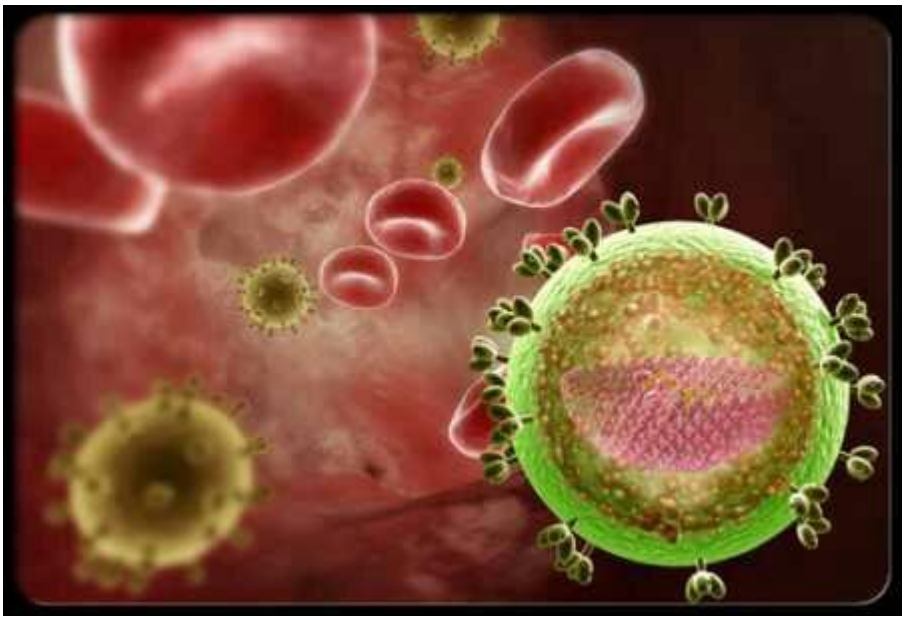


Figure:6 Human immuno deficiency virus

Human immunodeficiency virus (HIV) is the causative agent for AIDS. The most common type is known as HIV -1 and is the infectious agent that has led to the worldwide AIDS epidemic. These is also an HIV -2 that is much less common and less virulent, but eventually produces clinical findings similar to HIV-1. The HIV-1 type itself has a number of subtypes (A through H and O) which have differing geographic distributions but all produces AIDS similarly. HIV is a retrovirus that contains only RNA.

HIV is a sexually transmitted disease. Infection is aided by Langerhans cells in mucosal epithelial surfaces which can become infected. Infection is also aided by the presence of other sexually transmitted diseases that can produce mucosal ulceration and inflammation. The CD4+ T-lymphocytes have surface receptors to which HIV can attach to promote entry into the cell. The infection extends to lymphoid tissues which contain follicular dendritic cells that can become infected and provide a reservoir for continuing infection of CD4+ T-lymphocytes. HIV can also spread via blood products, most commonly with shared contaminated needles used by persons engaging in intravenous drug use. Mothers who are HIV infected can pass the virus on to their foetuses in utero or to infants via breast milk.

When HIV infects a cell,it must use its reverse transcriptase enzyme to transcribe its RNA to host cell proviral DNA. It is this proviral DNA that directs the cell to produce additional HIV virions which are released. When the CD4 lymphocyte count drops below 200/microliter, then the stage of clinical AIDS has been reached.

Lamivudine is an potential anti-HIV agent, used for the long term treatment of HIV-1 infection. It is approved by the U.S Food and Drug Administration (FDA). Lamivudine treatment in the present era. Dosage and duration of Lamivudine therapy should be individualized according to requirement and response of the patient.

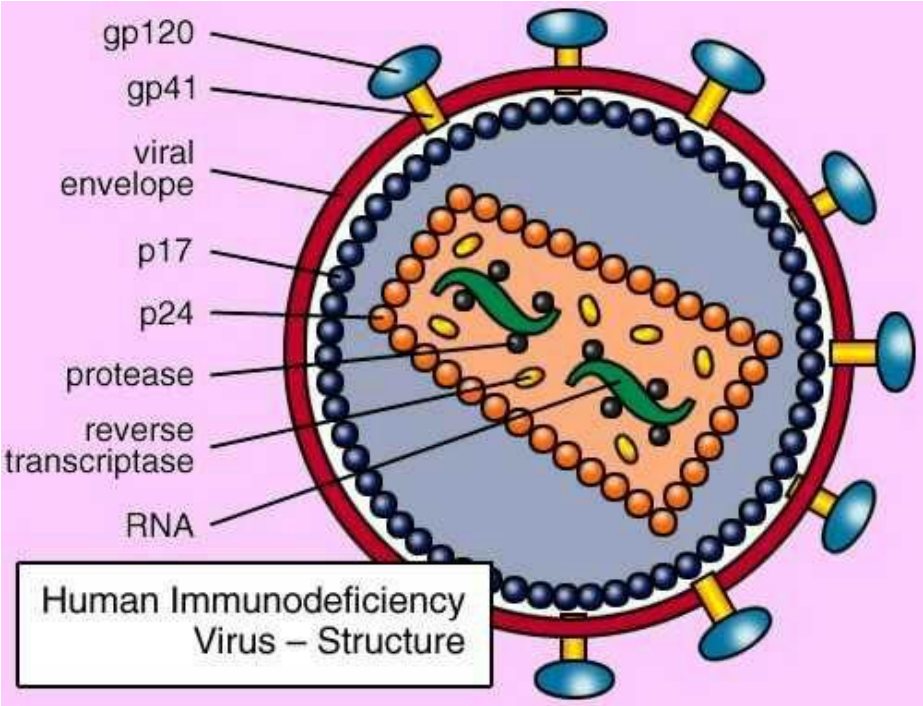


Figure:7 HIV Structure

HIV consists of a cylindrical center surrounded by a sphere-shaped lipid bilayer envelope. There are two major viral glycoproteins in this lipid bilayer, gp120 and gp41. The major function of these proteins is to mediate recognition of CD4+ cells and chemokine receptors, thereby enabling the virus to attach to and invade CD4+ cells. The inner sphere contains two single-strandedcopies of the genomic material, RNA, as well as multiple proteins and enzymes necessary for HIV replication and maturation: p24, p17, reverse transcriptase, integrase, and protease .

LIFE CYCLE OF HIV VIRUS

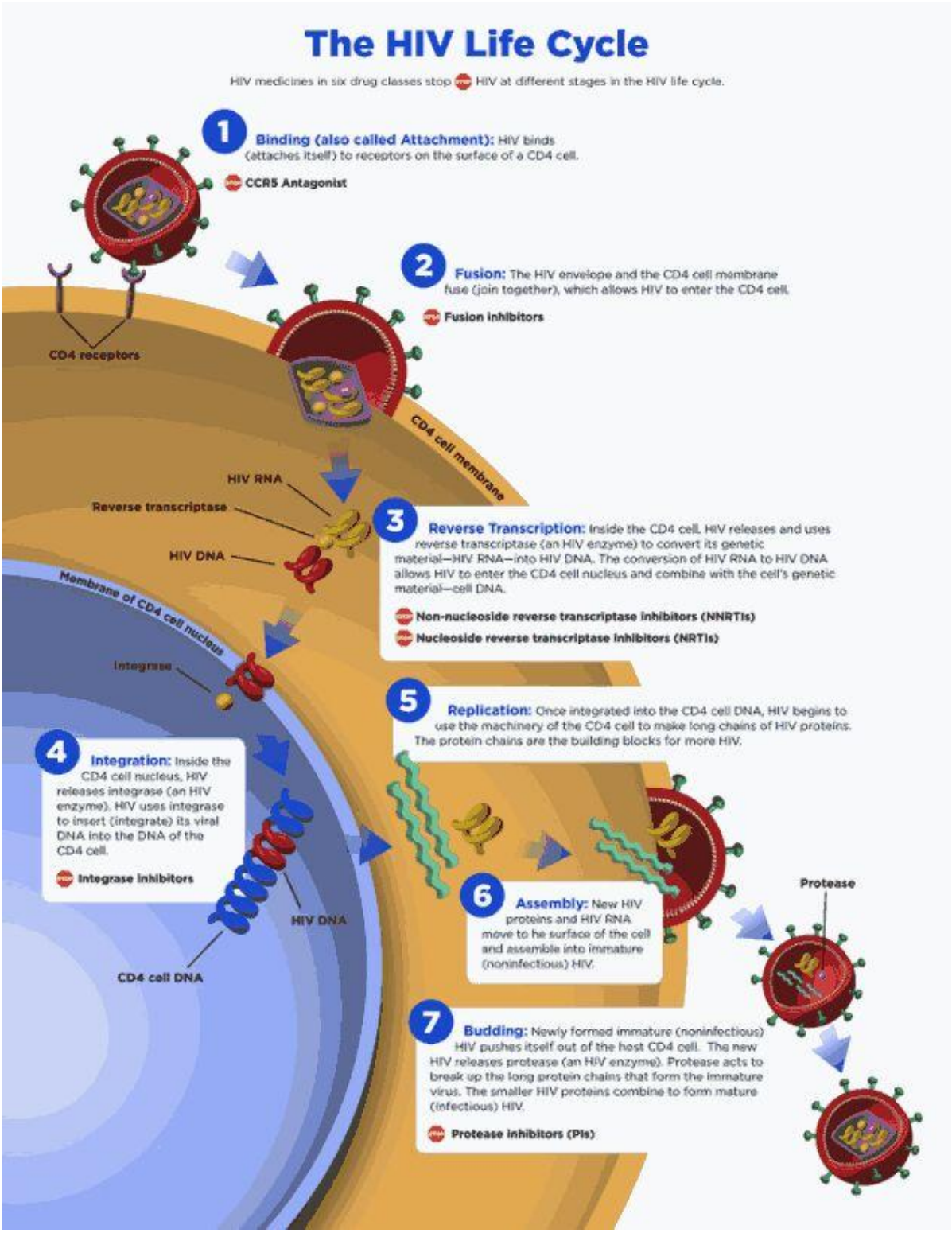


Figure:8 HIV Life cycle

TRANSMISSION OF HIV

- Unprotected sexual intercourse, especially receptive anal intercourse .
- A large number of sexual patners.
- Prior or current sexually transmitted diseases (STDs):
  - ✓ Gonorrhea and Chlamydia infections the HIV transmission risk 3-fold syphilis raises the transmission risk 7-fold.
  - ✓ Herpes genitalis raises the transmission risk up to 25-fold during an outbreak.
- Mucosal contact with infected blood or needle-stick injuries.
- Maternal HIV infection (for newborns, infants and children):

  Steps taken to reduce the risk of transmission at birth include cesarean delivery and prenatal antiretroviral therapy in the mother and antiretroviral therapy in the newborn immediately after birth.

HIV is not transmitted by

- Coughing, sneezing.
- Insect bites.
- Touching.
- Water and food.
- Hand shaking.
- Using telephones.
- Sharing cups, glasses, plates or other utensils.

Methods to reduce rates of HIV Transmission

- Treat HIV infection as an illness, not as a social stigma.
- Provide HIV testing and counseling to identify infected persons who can reduce their risk to others.
- Provide educational programs for children and adults which describe how to avoid.
- Immediate treatment of sexually transmitted diseases.
- Provide clean sterile needles for injection drug.
- Create HIV-infected pregnent women antiretroviral therapy to reduce perinatal HIV transmission.

- Consider pre-exposure Prophylaxis with antiretroviral drugs.

STAGES OF HIV INFECTION[38]

HIV infects cells in the immune system and the central nervous system. One of the main type of cells that HIV infects is the T helper lymphocyte. These cells play a crucial role in the immune system. A large reduction in the number of T helper cells seriously weakens the immune system.

HIV infects the T helper cell because it has the protein CD4 on its surface, which HIV uses to attach itself to the cell before gaining entry. This is the reason T helper cell is sometimes referred to as a CD4+ lymphocyte. Once it has found its way into a cell,HIV produces new copies of itself, which can then go on infect other cells.

HIV infection leads to a severe reduction in the number of T helper cells available to help fight disease. The number of T helper cells is measured by having a CD4 test and is referred to as the CD4 count. It can take several years before the CD4 count declines to the point that an individual needs to begin antiretroviral treatment. Without treatment, the CD4 count continues to decline to very low levels, at which point the individual is said to have progressed to AIDS.

HIV infection can generally be broken down into four distinct stages:

- Primary infection
- Clinically asymptomatic stage
- Symptomatic HIV infection
- Progression from HIV to AIDS

STAGE 1: Primary Infection

This stage of infection lasts for a few weeks and is often accompanied by a short flu-like illness. About 20% of the people the HIV symptoms are serious enough to consult a doctor, but the diagnosis of HIV infection is frequently missed. During this stage there is a large amount of HIV in the peripheral blood and the immune system begins to respond to the virus by producing HIV antibodies and cytotoxic lymphocytes. This process is known as seroconversion. If an HIV antibody test is done before seroconversionis complete then it may not be positive.

STAGE 2: Clinically asymptomatic stage

This stages lasts for an average of ten years and as its name suggests, is free from major symptoms, although there may be swollen glands. The level of HIV in the peripheral blood drops to very low levels is but people remain infectious and HIV antibodies are detectable in the blood, so antibody test will show a positive result.

STAGE3 : Symptomatic HIV infection

Over time immune system becomes severely damaged by HIV. This is thought to happen for three main reasons:

- ✓ The lymph nodes and tissues become damaged or 'burnt out' because of the years of activity.
- ✓ HIV mutates and become more pathogenic , in other words stronger and more varied, leading to more T helper cell destruction.
- ✓ The body fails to keep up with replacing the T helper cells that are lost.

Antiretroviral treatment is usually started once an individual CD4 count i.e the number of T helper cells drops to a low level, an indication that the immune system is deteriorating. Treatment can stop HIV from damaging the immune system, therefore, HIV –infected individuals on treatment usually remain clinically asymptomatic. However ,in HIV-infected individuals not receiving treatment or on treatment that is not working, the immune system fails and symptoms develop. Initially many of the symptoms are mild , but as the immune system deteriorates the symptoms worsen.

Symptomatic HIV infection is mainly caused by the emergence of certain infections that the immune system would normally prevent. This stage of HIV infection is often charecterised by multi-system disease and infections can occur in almost all body systems.

Treatment for the specific infection is often carried out, but the underlying cause is the action of HIV as it erodes the immune system. Unless HIV itself can be slowed down the symptoms of immune suppression will continue to worsen.

STAGE 4: Progression from HIV to AIDS

As the immune system becomes more and more damaged the individual may develop increasingly severe infections and cancers, leading eventually an AIDS diagnosis.

A clinical criteria is used by WHO diagnose the progression to AIDS, this differs slightly between adults and children under five. In adults and children (aged 5 or over) the progession to AIDS is diagnosed when any condition listen in clinical listed in clinical stage 4 is diagnosed or the CD4 count is less than 200 cells/mm or a CD4 percentage less than 15. In children younger than five, an AIDS diagnosis is based on having any stage 4 condition and/or a CD4 percentage less than 20 (children aged 12 – 35 months) and a CD4 percentage less than 25 (children less than 12 months).

SIGNS AND SYMPTOMS[31]

The patient with HIV may present with signs and symptoms of any of the stages of HIV infection. No physical findings are specific to HIV infection. Manifestations include the following:

- ✓ Acute seroconversion manifests as a flu like illness, consisting of fever, malaise and a generalized rash.
- ✓ The asymptomatic phase is generally benign.
- ✓ Lymphadenopathy is common.
- ✓ AIDS manifests as recurrent, severe and occationally life-threatening infections or malignancies.
- ✓ AIDS associated dementia/encephalopathy and HIV wasting syndrome (chronic diarrhea and weight loss with no indentifiable causes).
- ✓ Highly active antireteroviral therapy (HAART) is the principle method for preventing immune deterioration. Classes of antireteroviral agents include the following:
    - ✓ Nucleooside and Nucleotide reverse transcriptase inhibitors (NRTIs)
    - ✓ Non- Nucleotide reverse transcriptase inhibitors (NNRTIs)
    - ✓ Protease inhibitors (PIs)
    - ✓ Fusion inhibitors
    - ✓ HIV integrase strand transfer inhibitors

There has been improvement over time . Between 1996 and 2010 the rate of new HIV infections fell by 56 %. This trend is mainly due to a drop in infections in southern states; in other areas there has been no significant decline.

TREATMENT[6]

HIV is treated using a combination medicines to fight HIV infection . This is called antireteroviral therapy (ART). ART it is not cure , but it can control the virus so that you can live longer, healthier life and reduce the risk of transmitting HIV to others.

These HIV medicines prevent HIV from multiplying , which reduces the amount of HIV in our body gives immune system a chance to recover and fight off infections.

Table :5 Preferred and alternative anti-HIV regimens

Preferred regimens
2 NRTI + NNRTI (PI sparing)
1. Zidovudine + lamivudine + efavirenz
2 NRTI + PI
1. Zidovudine + lamivudine + lopinavir
Alternative regimens
2 NRTI + NNRTI (PI sparing)
1. Zidovudine + lamivudine + nevirapine
2. Lamivudine + stavudine + efavirenz
3. Lamivudine + stavudine + nevirapine
4. Lamivudine + abacavir + efavirenz
5. Lamivudine + abacavir + nevirapine
2 NRTI + PI
1. Lamivudine + zidovudine + indinavir
2. Lamivudine + stavudine + ritonavir
3. Lamivudine + abacavir + lopinavir/r
4. Lamivudine + abacavir + nelfinavir
*3 NRTI**
1. Zidovudine + lamivudine + abacavir

BLOOD DETECTION TEST :

- Enzyme- Linked Immunosorbent Assay/Enzyme Immunoassay (ELISA/EIA).
- Radio Immuno Precipitation Assay/Indirect Fluorescent Antibody Assay (RIP/IFA).
- Polymerase chain Reaction (PCR).
- Western blot Confirmatory test.

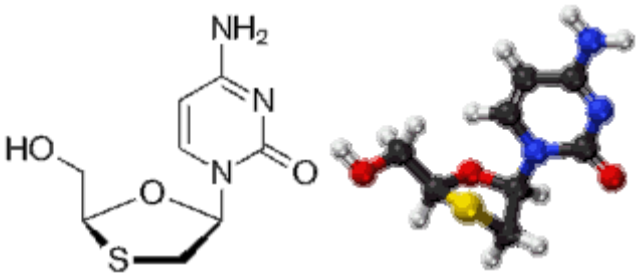
6. DRUG PROFILE[6,8,37,38]

Lamivudine is an antiviral drug. It is a novel cystosine nucleoside analog, reverse transcriptase inhibitor that has shown activity against Human Immunodeficiency Virus (HIV) types1 and 2 and hepatitis B virus.

Physicochemical profile

Official status **:** I.P, USP

Structure



Drug name	:	Lamivudine
Molecular formula	:	$C_8H_{11}N_3O_3S$
Molecular weight	:	229.26 g/mol
Melting point	:	186 □ C – 188 □ C
Chemical name	:	4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan- Yl]-1,2-dihydropyrimidin-2-one.
CAS number	:	134678-17-4
Category	:	Anti-HIV Agents Nucleoside Reverse Transcriptase Inhibitors
Description	:	A white or almost white powder
Solubility	:	Soluble in water , sparingly soluble in methanol, practically insoluble in acetone.
Bioavailability	:	86 %

Protien binding	:	< 36 %
Half life (t1/2)	:	5 – 7 Hours
Cmax	:	About 1.5 hours

Pharmacology

Lamivudine is an analogue of 2'3'-dideoxynucleoside 3'-thia cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. Lamivudine undergoes intracellular phosphorylation by kinases to form the pharmacologically active moiety 3TCTP that compete for incorporation into viral DNA . They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3'phosphodiester linkage essential for DNA chain elongation , and therefore, the viral DNA growth is terminated.

Pharmacodynamics

Lamivudine tri phosphate competes with deoxycytidine triphosphate for binding to reverse transcriptase and the incorporation of 3TC-triphosphate agent form in viral DNA results in chain termination.

Mechanism

During infection with HIV, the HIV virus multiplies within the body's cells. The viruses then are released from the cells and spread throught out the body where they infect other cells. In this manner, HIV infection spreads to new , uninfected cells that the body is continually producing and HIV infection perpetuated. When producing new viruses, the HIV virus must manufacture new DNA for each virus. Reverse transcriptase is the virus enzyme that forms this new DNA. Lamivudine is converted within the body to its active form, lamivudine triphospate. This active form is similar to a chemical, deoxycytidine triphosphate, that is used by reverse transcriptase to make new DNA. The reverse transcriptase uses lamivudine triphosphate instead of deoxycytidine triphosphate , and the lamivudine

triphosphate interfers with the reverse transcriptase. Lamivudine does not kill existing HIV virus and it is not a cure for HIV.

Pharmacokinetics

- **Absorption**

  Lamivudine was rapidly adsorbed after oral administration in HIV-infected patients. Following an oral dose, reaching maximal serum concentrations between 1 and 1.5 h, has good (> 80 %) absolute bioavailability. The influence of food on lamivudine absorption is not considered. Hence lamivudine can be given with or without food.

- **Distribution**

  86 % of lamivudine distributes into extravascular spaces. Volume of distribution was independent of dose and did not correlate with body weight. In pregnant women, lamivudine concentrations in maternal serum, amniotic fluid , umbilical cord and neonatal serum are comparable, indicating that the drug diffuses freely across the placenta. In postpartum women lamivudine is secreted into breast milk.

- **Metabolism**

  The only detected metabolite of lamivudine is trans-sulfoxide. Intracellularly it is metabolized to its active triphospate form by multiple kinase.

- **Elimination**

  Renal clearance is the major route of lamivudine elimination. Following oral administration 70 % dose is excreted unchanged in the urine and only 5- 10 % undergoes hepatic metabolism to form a trans-sulfoxide which is then also renally eliminated. Lamivudine is excreted in human breast milk.

Dosage

Lamivudine

Tablets : 100,150 and 300 mg

Oral solution : 5,10 mg/ml

- **Oral solution**

  Neonates (< 1 month) - 2 mg/kg twice daily.

  Infants and children (< 16 years) - 4mg/kg twice daily.

- **Tablets**

  Adults (> 16 years) : 150 mg every 12 hours

- **Combination**
  - 150 mg Lamivudine and 300mg Zidovudine
  - 300mg Abacavir and 150mg Lamivudine and 300 mg Zidovudine

ADVERSE EFFECTS

- Head ache
- Dizziness
- Nausea
- Vomiting
- Chills
- Cough
- Loss of appetite
- Fatigue
- Abdominal pain
- Anorexia
- Weight loss
- Diarrheoa
- Lactic acidosis
- Changes in body fat.

Precautions

Precautions has to be taken in people having kidney or liver disease or history of pancreatitis, diabetes, pregnancy and nursing mothers.

Contraindications

Drug resistance to Anti-reteroviral therapy, severe liver disease , acute and chronic inflammation of the pancreas, kidney disease , Nursing mothers , increased blood Acidity due to high levels of Lactic Acid, Overweight.

Drug interactions

Tobramycin - Increased risk of nephrotoxicity

Valganiciclovir - risk of hematologic toxicity

Zalcitabine - reduce the efficacy of lamivudine

Trimethoprim - Increases concentration of Lamivudinein blood

Ribavir - Increases the risk of lamivudine side effects.

Uses

To prevent HIV infections

At low doses used to treat chronic Hepatitis B.

SORBITAN MONOPALMITATE[8]

SYNONYMS

Arbunol S-40; Alracel 40; Crill 2; Liposorb P; Montane 40; Sorbitanpalmitate; Span 40.

CHEMICAL NAME

Sorbitan Mono Hexadecanoate

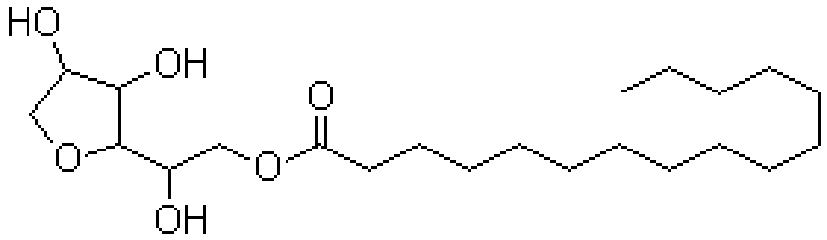
EMPIRICAL FORMULA

$C_{22}H_{42}O_6$

MOLECULAR WEIGHT

403

STRUCTURE



DESCRIPTION

Creamy solid

PROPERTIES

- Acid value - 3- 7
- Hydroxyl value - 270- 303
- Saponification value - 142- 152
- Melting point - 43- 48°C
- Density (g/cm^3) - 1.0 g/cm^3
- HLB value - 6.7

FUNCTIONAL CATEGORY

- Emulsifying agent
- Non- ionic surfactant

- Solubiizing agent
- Wetting agent
- Dispersing/ Suspending agent

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well- closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25 mg/ kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOSTEARATE[8]

SYNONYM

Arbunol S- 60; Alracel 60; Crill 3; Liposorb S-K; Montane 60; Sorbitan stearate; Span 60; Tego SMS.

CHEMICAL NAME

Sorbitan Mono- Octadecanoate

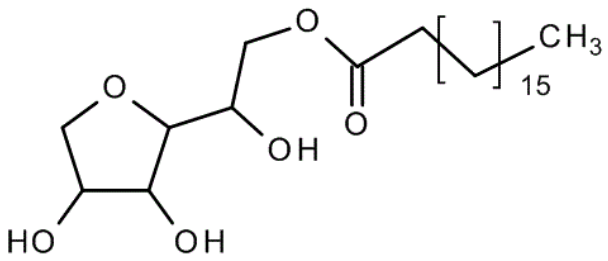
EMPIRICAL FORMULA

$C_{24}H_{46}O_6$

MOLECULAR WEIGHT

431

STRUCTURE



DESCRIPTION

Cream solid

PROPERTIES

- Acid value - 5- 10
- Hydroxyl value - 235- 260
- Saponification value - 147- 157
- Melting point - 53- 57°C
- HLB value - 4.7

FUNCTIONAL CATEGORY

- Emulsifying agent
- Non- ionic surfactant

- Solubilizing agent
- Wetting agent
- Dispersing/ Suspending agent

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well- closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25 mg/ kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

POLYSORBATE 60[8]

SYNONYM

Atlas 70K; Crillet 3; Glycosporse S- 20; Liposorb S- 20; Polyoxyethylene 20 stearate; Sorbitanmonooctadecanoate; Tween 60; Tween 60 K; Tween 60 VS.

CHEMICAL NAME

Polyoxyethylene 20 SorbitanMonostearate

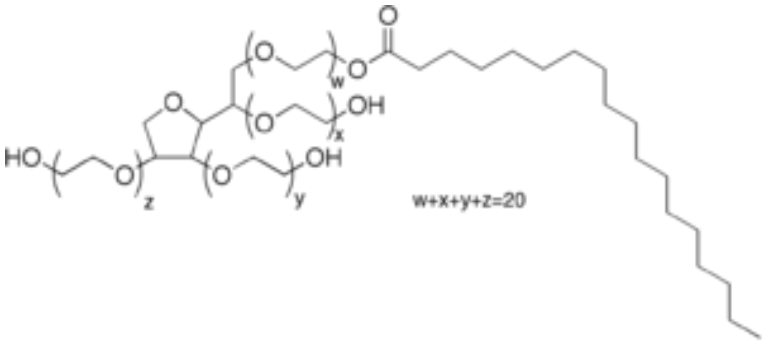
EMPIRICAL FORMULA

$C_{64}H_{126}O_{26}$

MOLECULAR WEIGHT

1312

STRUCTURE



DESCRIPTION

Yellow oily liquid

PROPERTIES

- Acid value - 2.0
- Hydroxyl value - 81- 96
- Saponification value - 45- 55
- Melting point - 53- 57°C
- HLB value - 14.9
- Solubility -soluble in ethanol and water.

FUNCTIONAL CATEGORY

- Emulsifying agent
- Non- ionic surfactant
- Solubilizing agent
- Wetting agent

STABILITY

- Stable to electrolytes and weak acids and bases
- Gradual saponification occurs with strong acids and bases
- It is hygroscopic and should be examined for water content prior to use and dried if necessary
- Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

STORAGE

It should be stored in a well- closed container protected from light, in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25 mg/ kg body weight and moderately toxic by i.v. route

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

CHOLESTEROL[8]

SYNONYM

Cholesterolum; Cholesterin

CHEMICAL NAME

Cholest- 5-en-3β- ol

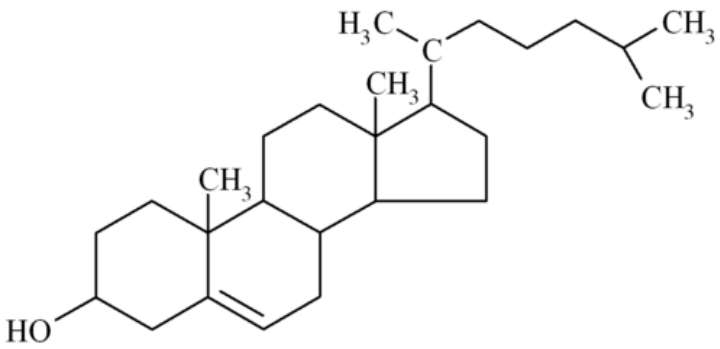
EMPIRICAL FORMULA

$C_{27}H_{46}O$

MOLECULAR WEIGHT

386.67

STRUCTURE



FUNCTIONAL CATEGORY

- Emulsifying agent
- Emollient

DESCRIPTION

- Cholesterol occurs as white or faintly yellow, almost odourless, pearly leaflets, needles, powder or granules.
- On prolonged exposure to light and air, it acquires a yellow to tan colour.

PROPERTIES

- Boiling point - 360°C
- Density - 1.052 g/ cm^3 for anhydrous form
- Melting point - 147- 150°C
- Solubility - Soluble in acetone and vegetable oils

  Practically insoluble in water, Soluble in chloroform: methanol mixture.

STABILITY AND STORAGE CONDITIONS

It is stable, and should be stored in a well- closed container and protected from light.

SAFETY

It is generally regarded as an essentially non- toxic and non- irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

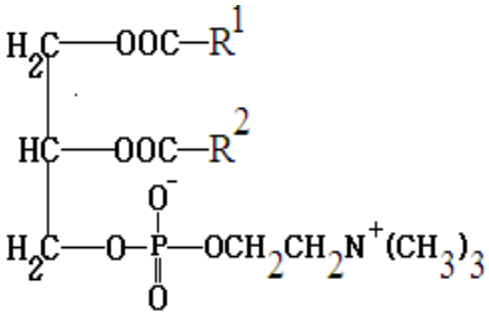
Rubber or plastic gloves, eye protection and a respirator are recommended.

LECITHIN[8]

SYNONYM

Egg lecithin; mixed soybean phosphatides; ovolecithin; ; soybean lecithin; soybean phospholipids; vegetable lecithin.

CHEMICAL STRUCTURE:



CHEMICAL NAME:

1,2-diacyl-sn-glycero-3-phosphocholine.

DESCRIPTION:

Lecithins vary greatly in their physical form, from viscous semisolids to powders, depending upon the free fatty acid content. They may also vary in color from brown to lightyellow. When they are exposed to air, rapid oxidation occurs, also resulting in a dark yellow or brown color. Lecithins are soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids. They are practically insoluble in cold vegetable and animal oils, polar solvents, and water. When mixed with water, however, lecithins hydrate to form emulsions.

PROPERTIES:

Density	: 0.5 g/cm3 for powdered lecithin.
Iodine number	: 82–88 for powdered lecithin.
Isoelectric point	: ≈3.5
Saponification value	: 196.

USES:

Emollient; emulsifying agent; solubilizing agent, vesicle stabilizing agent.

MALTODEXTRIN[8]

Synonyms

C*Dry MD,Malta*Gran;Rice,Tapi.

Chemical Name

Maltodextrin

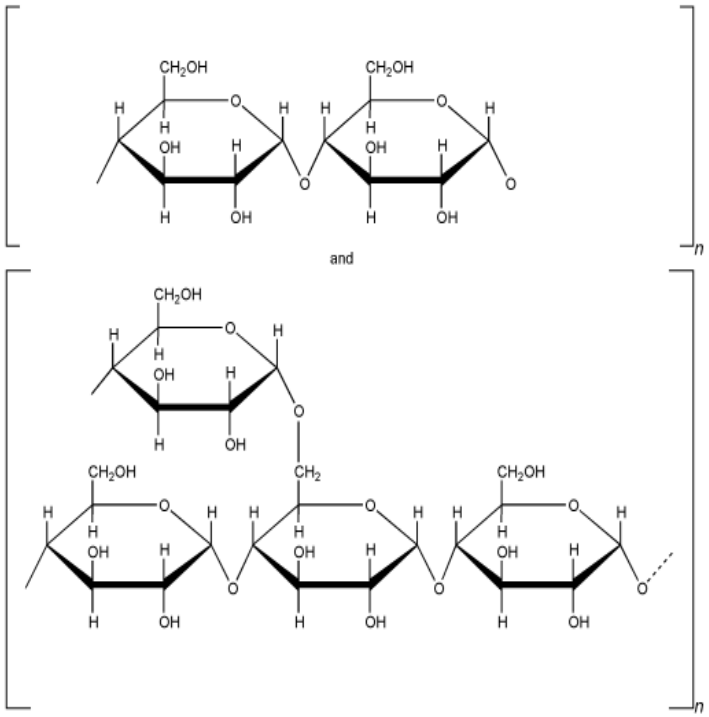
Empirical Formula

$(C_6H_{10}O_5)_n \cdot H_2O$

Molecular Weight

900–9000

Stucture



Functional Category

Coating agent; tablet and capsule diluents, tablet binder; viscosity-increasing agent.

Description

Maltodextrin occurs as a non sweet, odorless, white powder or granules. The solubility, hygroscopicity, sweetness, and compressibility of maltodextrin increase as the DE increases. The USPNF 23 states that it may be physically modified to improve its physical and functional characteristics.

Solubility:

Freely soluble in water; slightly soluble in ethanol (95%). Solubility increases as DE increases.

Stability and Storage Conditions

Maltodextrin is stable for at least 1 year when stored at a cool temperature (<30°C) and less than 50% relative humidity. Maltodextrin solutions may require the addition of an antimicrobial preservative. Maltodextrin should be stored in a well-closed container in a cool, dry place.

Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection is recommended. Maltodextrin should be handled in a well ventilated environment and excessive dust generation should be avoided.

Use

Diluent in single unit dosage form, coating agents, binding agent.

9. RESULTS AND DISSCUSION

DRUG EXCIPIENT COMPATIBILITY STUDIES

The possibilities of drug- excipient (cholesterol, nonionic surfactant) interactions were investigated by recording the FT- IR spectrum. The FT- IR spectra of the drug and the formulations are shown below.

Figure11: FT- IR spectra of Lamivudine standard

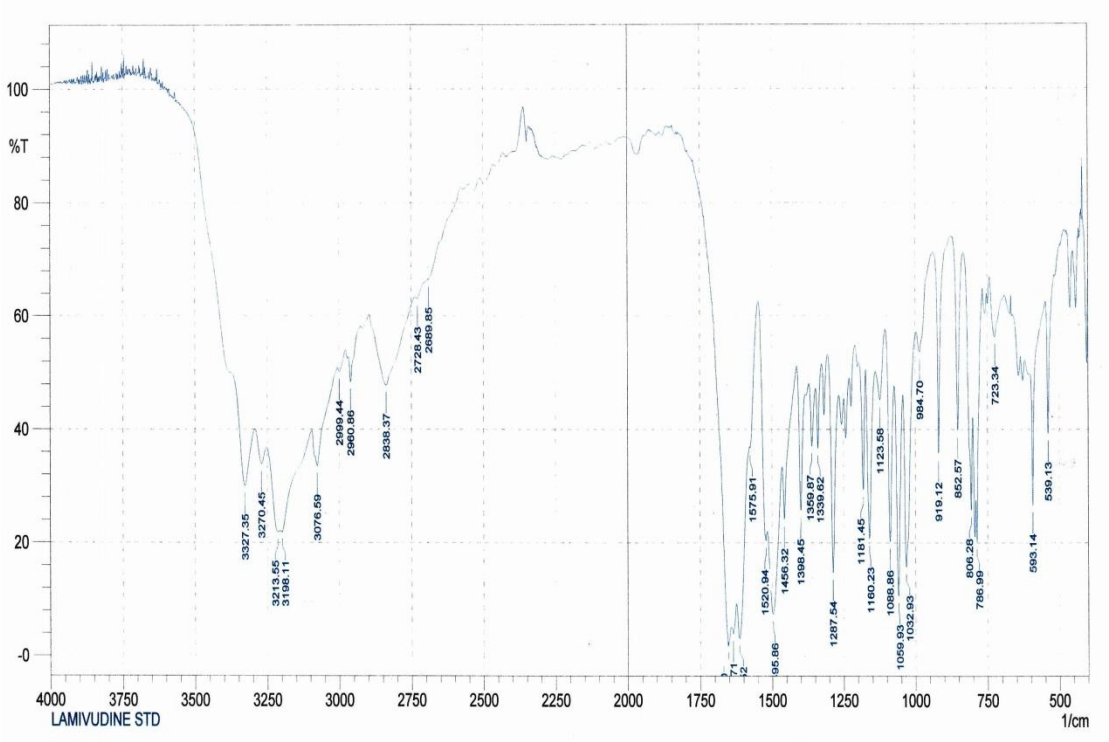


Table 11: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

Figure12: FT- IR spectra of Lamivudine with Span 40

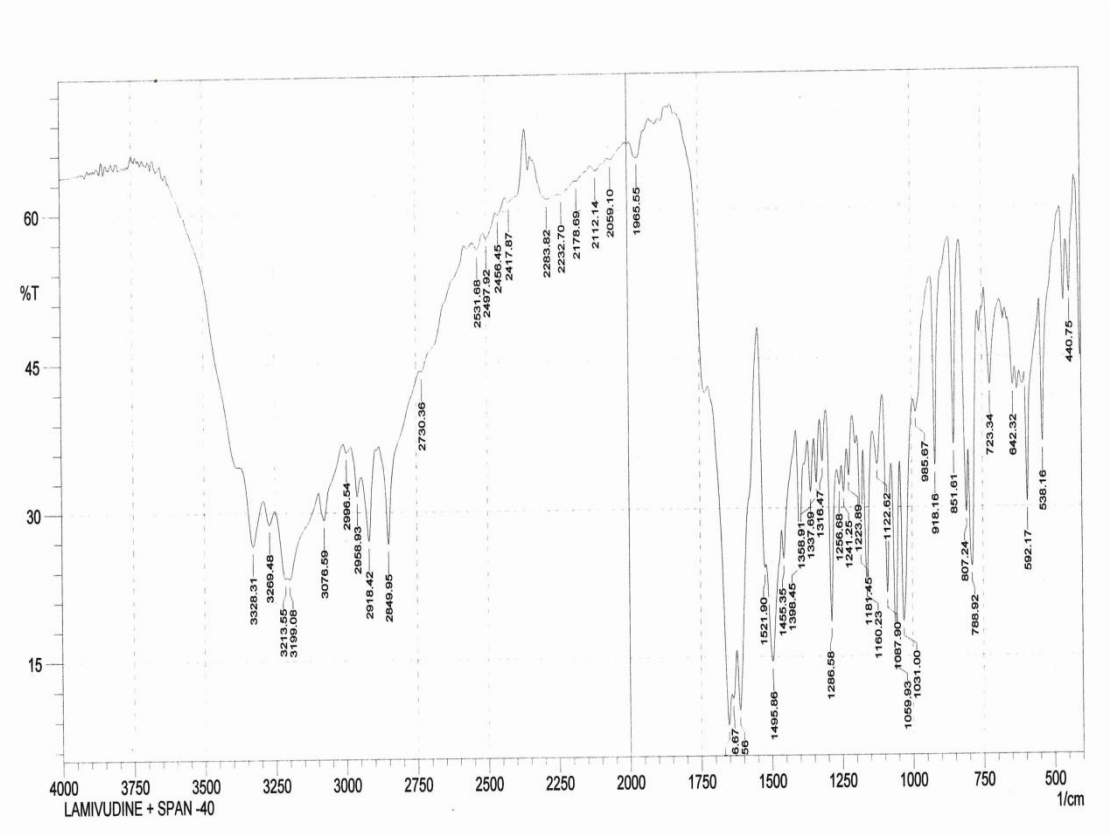


Table 12: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and Span 40. It is concluded that there is no interaction between the drug and excipients i.e. the drug is compatible with Span 40[27].

Figure113: FT- IR spectra of Lamivudine with Span 60

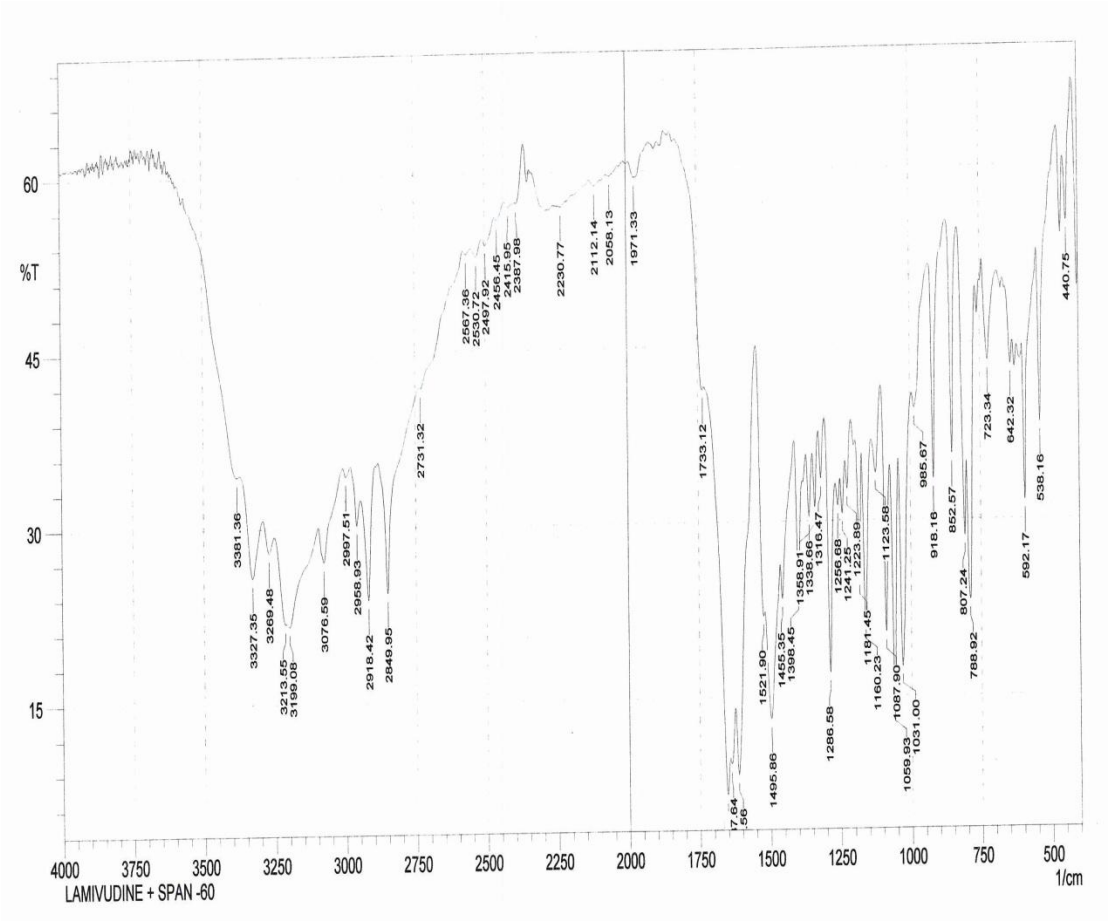


Table 13: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and Span 60. It is concluded that there is no interaction between the drug and excipients . The drug is compatible with Span 60[27].

Figure14: FT- IR spectra of Lamivudine with Tween 60

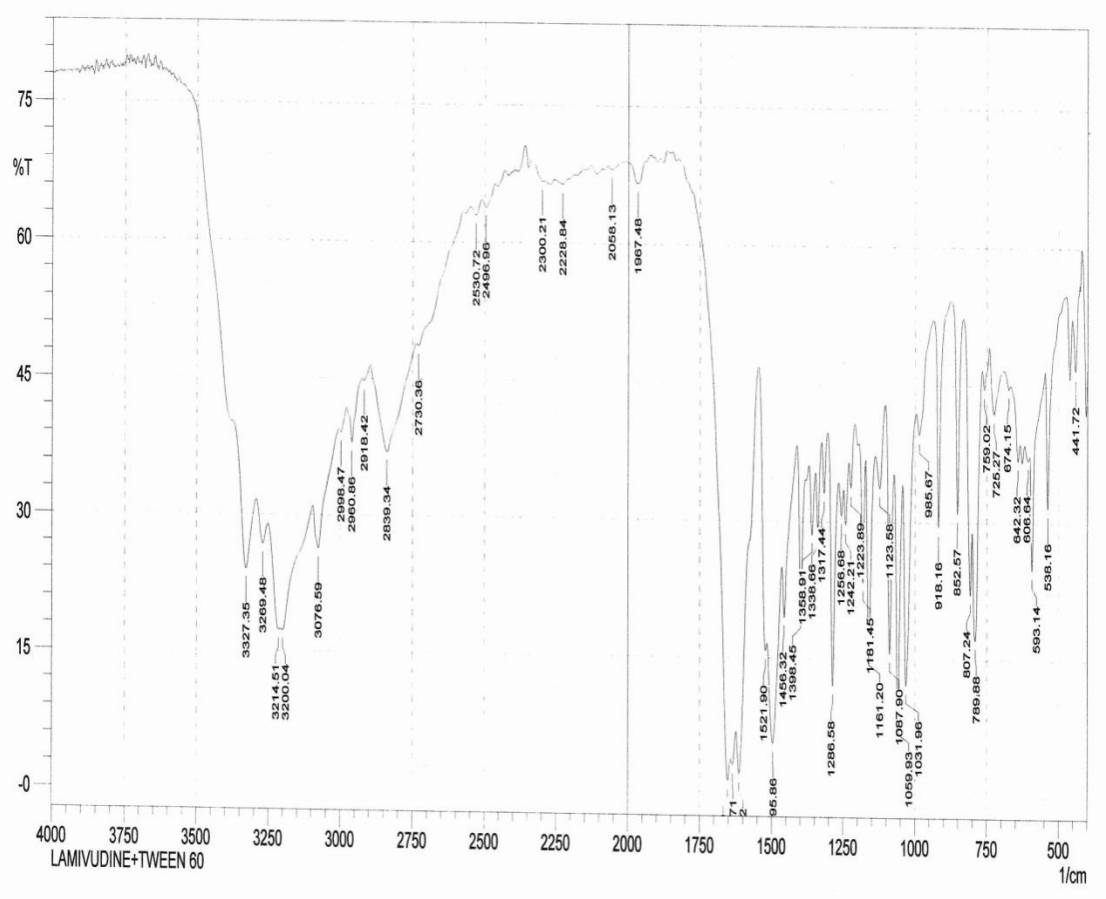


Table 14: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and Tween 60. It is concluded that there is no interaction between the drug and excipients . The drug is compatible with Tween 60[27].

Figure15: FT- IR spectra of Lamivudine with Cholesterol

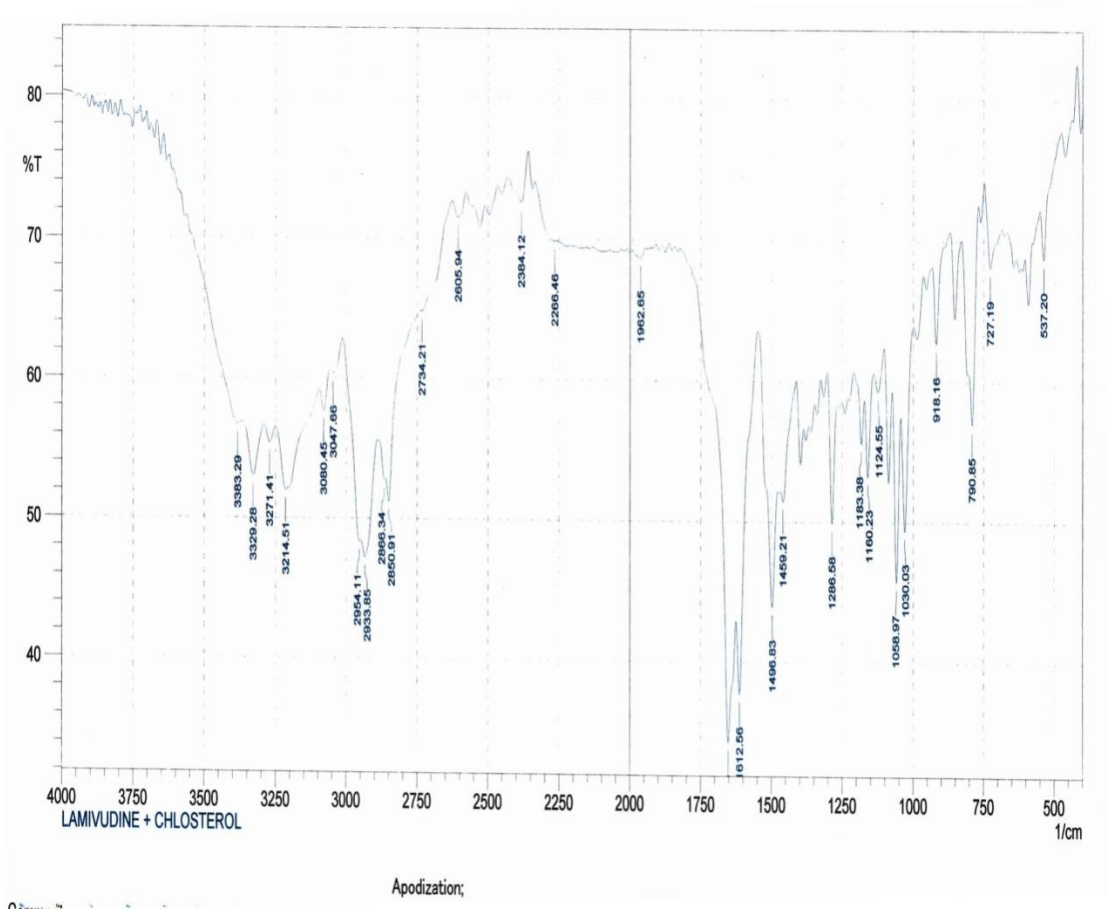


Table 15: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and cholesterol. It is concluded that there is no interaction between the drug and excipients . The drug is compatible with cholesterol [27].

Figure16: FT- IR spectra of Lamivudine with Maltodextrin

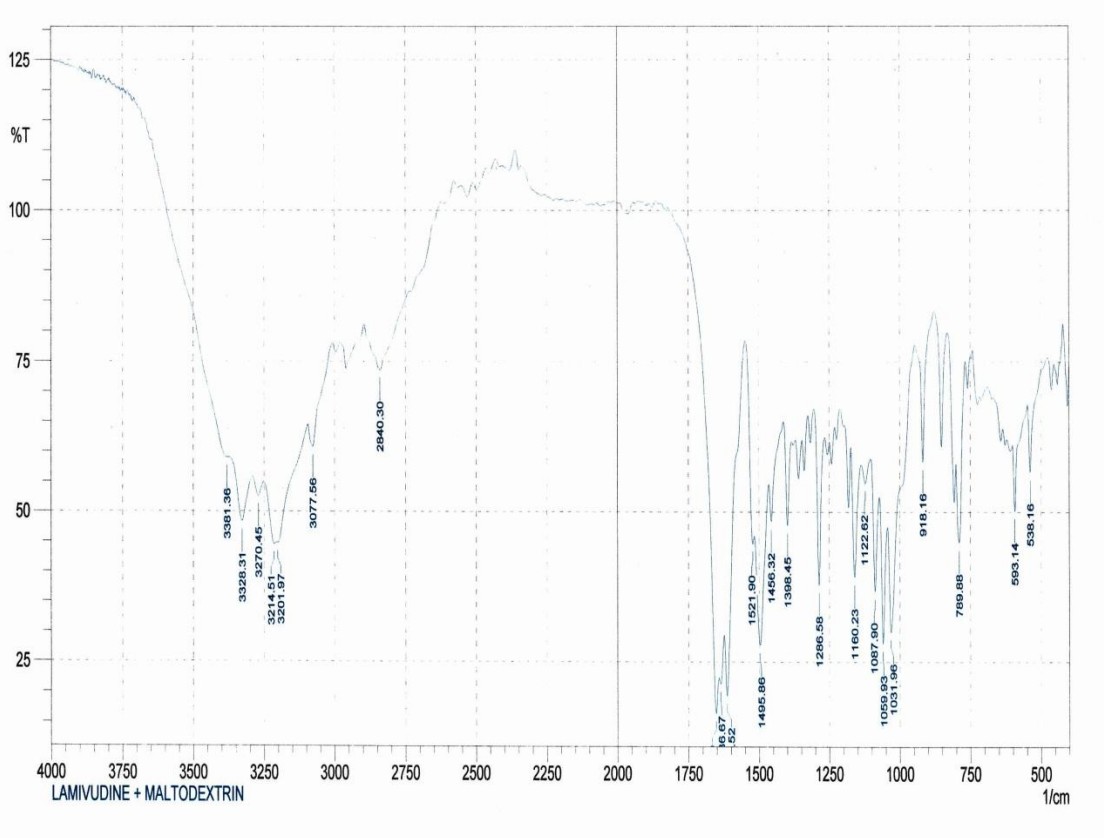


Table 16: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and maltodextrin. It is concluded that there is no interaction between the drug and excipients .The drug is compatible with maltodextrin [27].

Figure17: FT- IR spectra of FS3

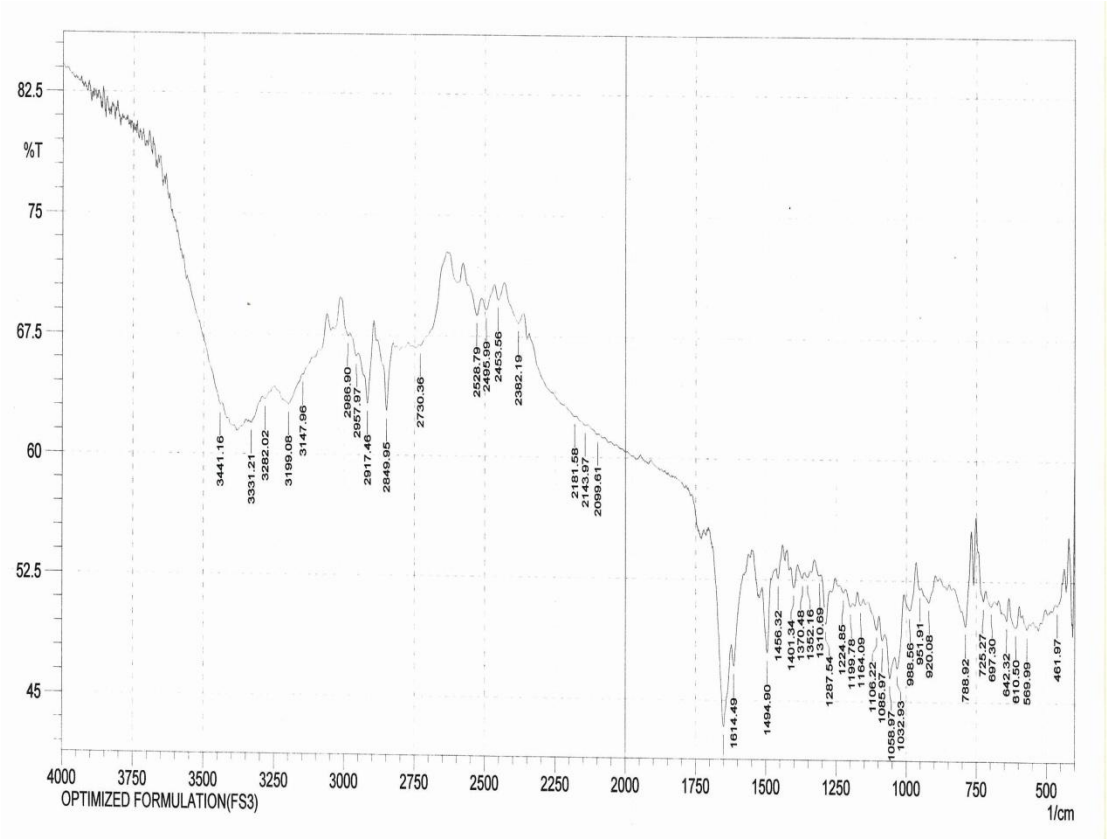


Table 17: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	3327.35	-NH stretching
2	3270.45	-O- H stretching
3	2960.86	Aliphatic C-H stretching
4	3076.59	Aromatic C-H streching

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and proniosomal formulation. It is concluded that there is no interaction between the drug and excipients[27].

STANDARD CURVE FOR LAMIVUDINE

The UV Spectrophotometric method was used to analyse Lamivudine. The absorbance of the drug in distilled water was measured at a wavelength of 270 nm. The results are given in Table18 and Figure 18.

Table18: Data for Calibration Curve of Lamivudine

S. No.	Concentration (µg/ ml)	Absorbance at 270 nm
1	0	0
2	2	0.076±0.011
3	4	0.156±0.01
4	6	0.249±0.0098
5	8	0.332±0.0094
6	10	0.428±0.019

*Mean± SD (n=3)

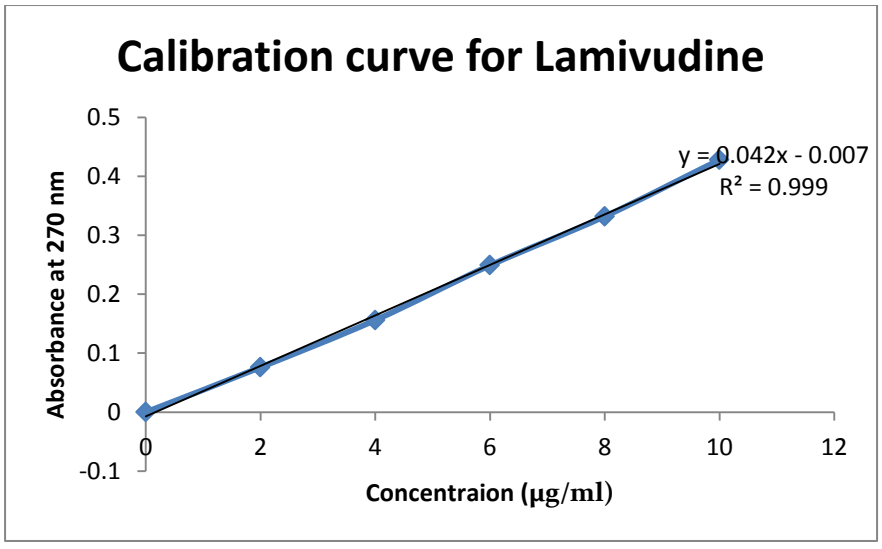


Figure 17: Calibration curve

The standard curve of Lamivudine in distilled water was linear in 2 to 10 µg/ ml concentrations.

EVALUATION RESULTS OF LAMIVUDINE PRONIOSOMES BY SLURRY METHOD

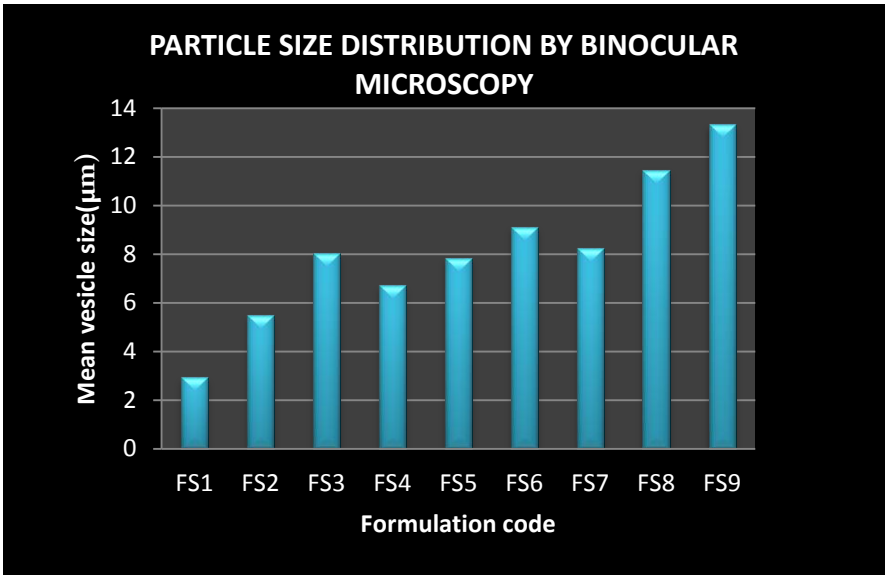
Table 19: Formulation table

FORMULATION CODE	NON – IONIC SURFACTANT	DRUG (mg)	MALTODEXTRIN: SURFACTANT: CHOLESTEROL (mg)
FS1	SPAN 40	300	100:100:100
FS2		300	100:200:100
FS3		300	100:300:100
FS4	SPAN 60	300	100:100:100
FS5		300	100:200:100
FS6		300	100:300:100
FS7	TWEEN 60	300	100:100:100
FS8		300	100:200:100
FS9		300	100:300:100

Table 20 :Mean vesicle size, drug content and entrapment efficiency of Lamivudine proniosomes by slurry method

FORMULATION CODE	NON – IONIC SURFACTANT	MEAN VESICLE SIZE (µm)	% DRUG CONTENT (% w/w)	% ENTRAPMENT EFFICIENCY (% w/w)
FS1	SPAN 40	2.94	95.92	76.24
FS2		5.49	98.85	71.13
FS3		8.04	90.67	**95.02**
FS4	SPAN 60	6.69	97.67	82.05
FS5		7.83	91.62	83.33
FS6		9.12	84.75	85.54
FS7	TWEEN 60	8.22	101.1	45
FS8		11.46	74.25	55.86
FS9		13.32	97.10	86.85

Figure19 : Particle size distribution of Lamivudine proniosome powder



Lamivudine proniosomes prepared using Span 40,Span 60 and Tween 60 at various concentrations . The particle size of the hydrated proniosomes powder was measured using an binocular microscope with calibrated eyepiece micrometer. From every batch about 100 vesicles were measured individually and the average diameter was calculated. The average vesicular size of hydrated niosomes was in the range of 2.94 to 8.04 µm for FS1 to FS3; 6.69 to 9.12µm for FS4 to FS6 and 8.22 to 13.32 µm for FS7 to FS9 formulations. The size distribution was in the range of 2 to 14 µm. The result suggests that hydrated proniosomes prepared were of uniform size and spherical in shape. The microphotographs of all hydrated proniosomal formulations reveal that the niosomes were spherical in their shape. From these results the vesicle size were found to increase with increase in the concentration of surfactant[40].

Figure 20: Drug Content of Lamivudine proniosome powder

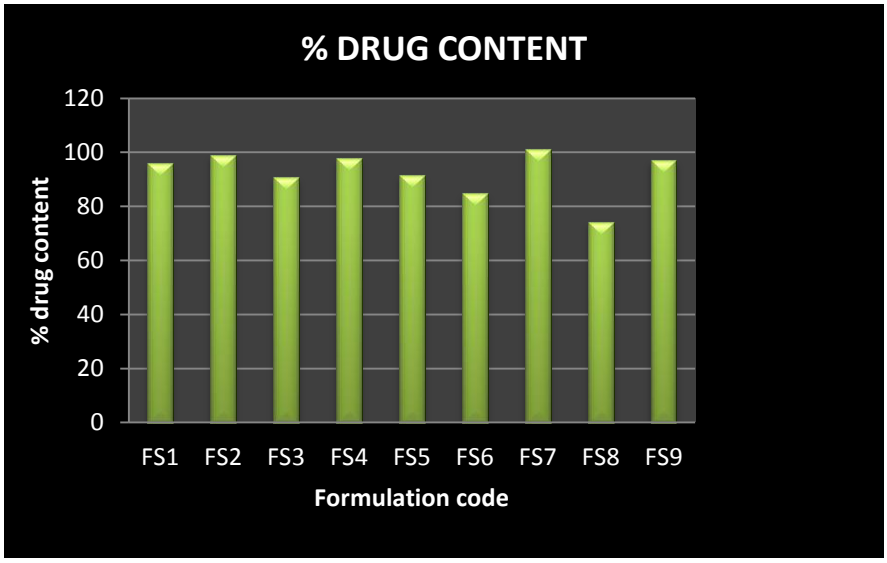


Figure 21: Entrapment Efficiency of Lamivudine proniosome powder

The Lamivudine proniosomes were prepared at various surfactant concentrations. The percentage entrapment efficiency was found to be in the range of 76.24 to 95.02 for FS1 to FS3; 82.05 to 85.54 for FS4 to FS6; 45 to 86.85 for FS7 to FS9 formulations. Higher surfactant concentration of span 60, span 40 and Tween 60 shows higher entrapment efficiency which might be due to the high fluidity of the vesicles [27]. The entrapment efficiency was increase with increasing surfactant

concentration. The larger vesicle size may also contribute to the higher entrapment efficiency. The highest entrapment efficiency was observed with Span 40 (FS3) formulation . This may be due to the higher HLB value of Span 40 compared to Span 60. Tween 60 niosomes showed comparatively very low entrapment efficiency which may be due to the very high HLB value 14.9 of Tween 60. The results coinside with earlier reports[27,29,80.]

The order of non ionic surfactants that resulted in better entrapment efficiency was as follows:

Span 40> Span 60> Tween 60

***IN- VITRO* RELEASE STUDY**

The *in-vitro* release study of Lamivudine proniosomes was carried out using dialysis membrane in an open ended tube- beaker assembly using distilled water as the diffusion medium.

Table 21: *In- vitro* release of proniosomes containing Span 40 in different ratios

Time in hours	Cumulative % drug release			
	Control	**FS1**	**FS2**	**FS3**
0	0	0	0	0
0.5	-	34.46	35.08	8.8
1	64.23	47.61	49.95	14.22
1.5	-	58.08	53.26	18.53
2	85.53	64.46	66.31	21.82
2.5	-	69.58	73.06	26.90
3	105.5	74.98	78.11	30.58
3.5	-	77.73	82.31	33.35
4	-	84.28	89.09	36.69
4.5	-	90.98	98.45	40.55
5	-	97.58	102.88	44.39
6	-	99.23	-	48.68
7	-	-	-	-
8	-	-	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
18	-	-	-	58.94
19	-	-	-	64.47
20	-	-	-	72.86
21	-	-	-	78.46
22	-	-	-	83.65
23	-	-	-	86.66
24	-	-	-	**93.53**

Table 22: *In- vitro* release of proniosomes containing Span 60 in different ratios

Time in hours	Cumulative % drug release			
	Control	**FS4**	**FS5**	**FS6**
0	0	0	0	0
0.5	-	18.37	15.25	21.25
1	64.23	22.36	23.31	25.58
1.5	-	27.13	30.91	29.16
2	85.53	31.16	37.21	31.48
2.5	-	34.83	44.75	34.83
3	105.5	39.58	48.85	39.66
3.5	-	42.06	50.76	43.98
4	-	45.35	60.78	49.45
4.5	-	63.58	63.06	54.45
5	-	58.23	65.63	57.18
6	-	66.43	72.21	63.21
7	-	70.96	77.21	68.40
8	-	76.71	87.21	75.68
9	-	85.40	89.63	84.35
10	-	93.15	95.61	89.91
11	-	100.1	99.56	94.21
12	-	-	-	**97.96**
24	-	-	-	-

Table 23: *In- vitro* release of proniosomes containing Tween60 in different ratios

Time in hours	Cumulative % drug release			
	Control	**FS7**	**FS8**	**FS9**
0	0	0	0	0
0.5	-	30.16	24.75	18.91
1	64.23	47.51	36.15	22.36
1.5	-	62.45	41.86	27.15
2	85.53	74.33	49.33	31.75
2.5	-	80.01	59.31	36.03
3	105.5	87.03	63.58	39.81
3.5	-	89.25	66.93	42.86
4	-	94.38	77.33	47.58
4.5	-	98.16	86.01	52.71
5	-	-	92.01	57.73
6	-	-	102.1	64.61
7	-	-	-	76.83
8	-	-	-	83.46
9	-	-	-	90.71
10	-	-	-	**98.03**
11	-	-	-	-
12	-	-	-	-
24	-	-	-	-

Figure 22: *In- vitro* release of proniosomes containing Span 40 in different ratios

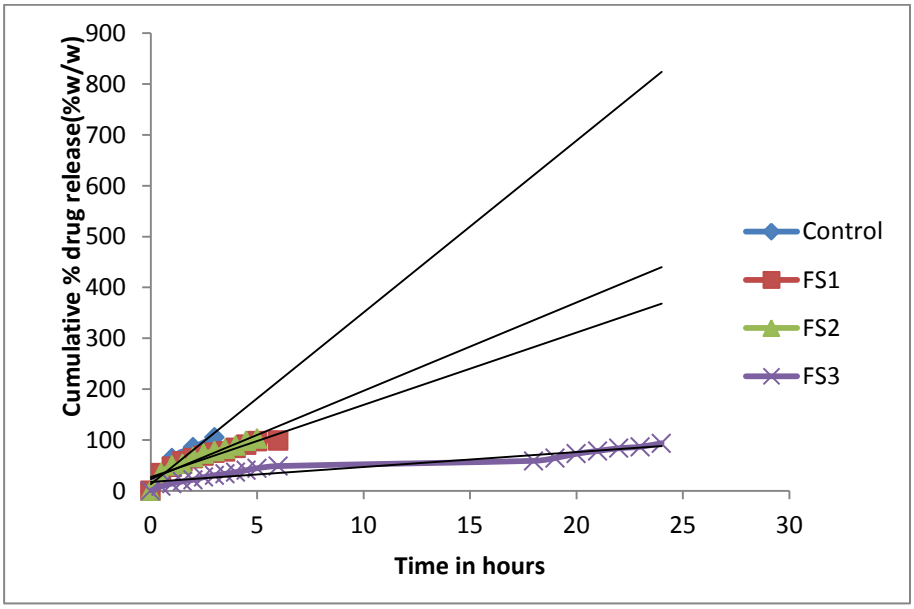


Figure23: *In- vitro*release of proniosomes containing Span 60 in different ratios

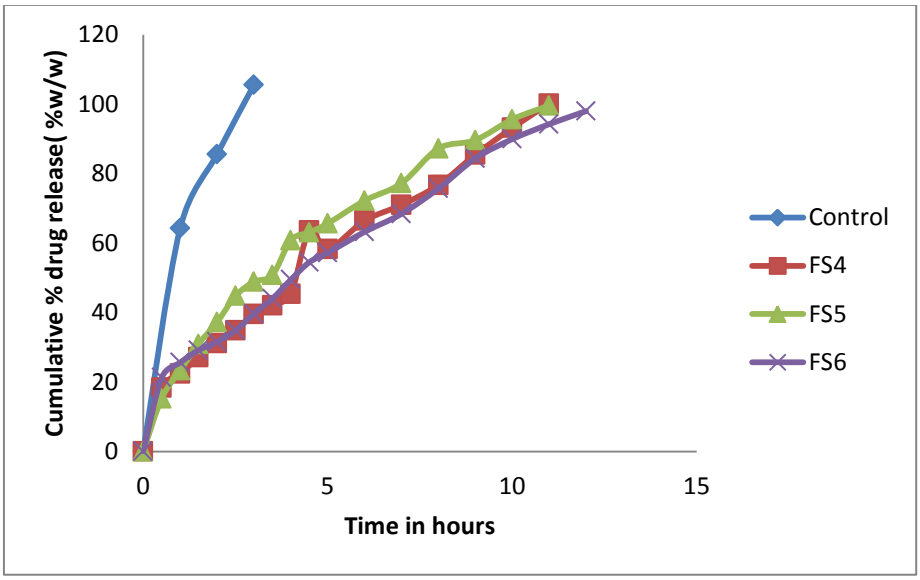


Figure 24: *In- vitro* release of proniosomes containing Tween 60 in different ratios

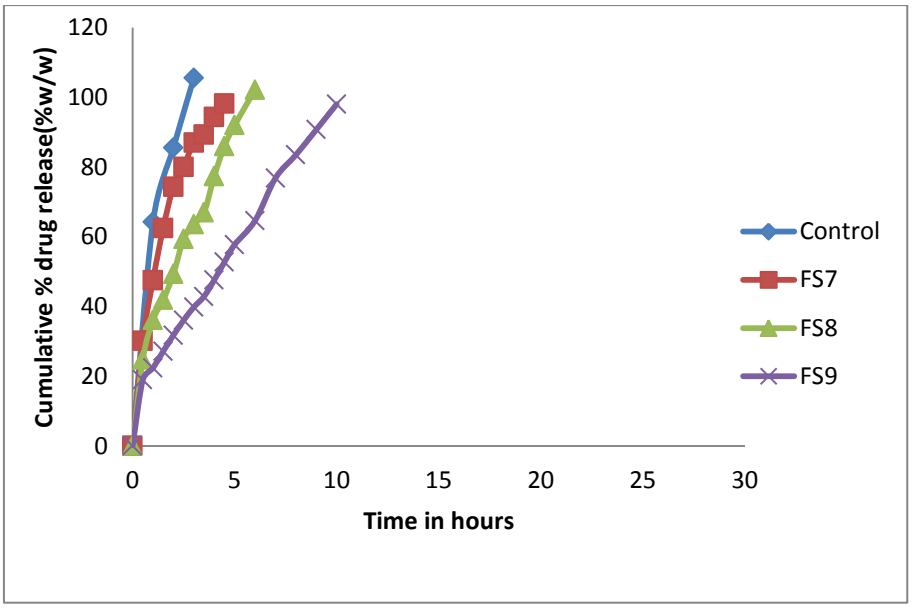
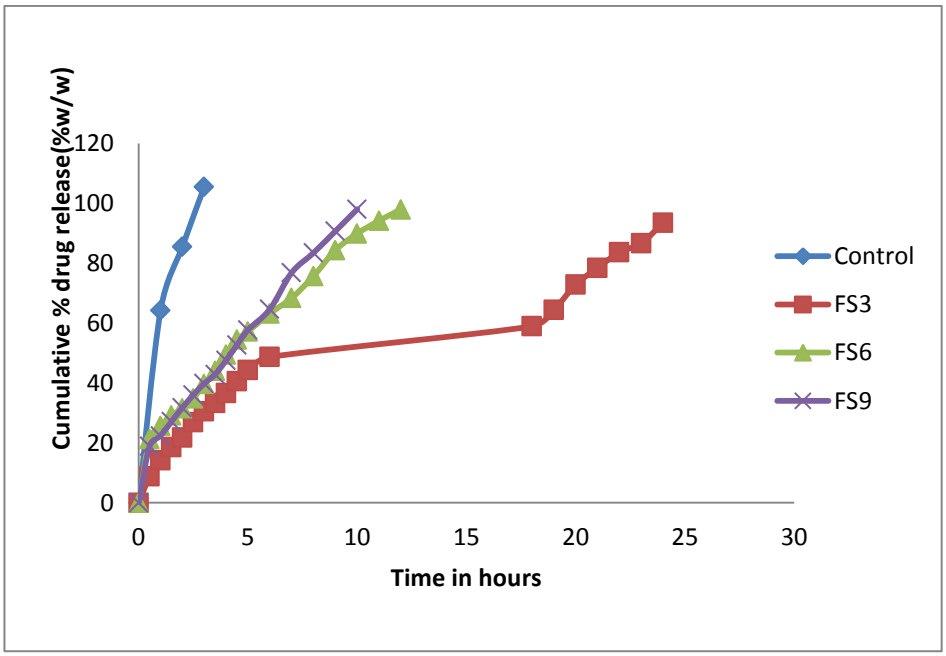


Figure 25: Comparison of *in- vitro* release of proniosomes with highest entrapment efficiency



The lamivudine proniosomes were prepared at various concentration of surfactants. The cumulative percentage drug release was found to be in the range of 34.46 to 99.23 for FS1 to FS3; 97.96 to 100 for FS4 to FS6; 98.03 to 102 for FS7 to FS9 formulations. Higher surfactant concentration of Span 60, Span 40 and Tween 60 showed higher entrapment efficiency and more sustained release. The more sustained drug release was observed with Span 40 (FS3) . This may be due to the higher entrapment efficiency. The FS3 formulation showed sustained release of 93.53% over 24 hours. The results coinside with earlier reports [27,29,80].

All the proniosomal formulations showed sustained release when compared with control.

The cumulative % drug release at 24 hours for different ratios of the three non- ionic surfactants was in the following order:

Span 40	**:**	**FS1>FS2>FS3**
Span 60	**:**	**FS4>FS5>FS6**
Tween 60	**:**	**FS7>FS8>FS9**

EVALUATION RESULTS OF LAMIVUDINE PRONIOSOMES BY COACERVATION PHASE SEPARATION METHOD

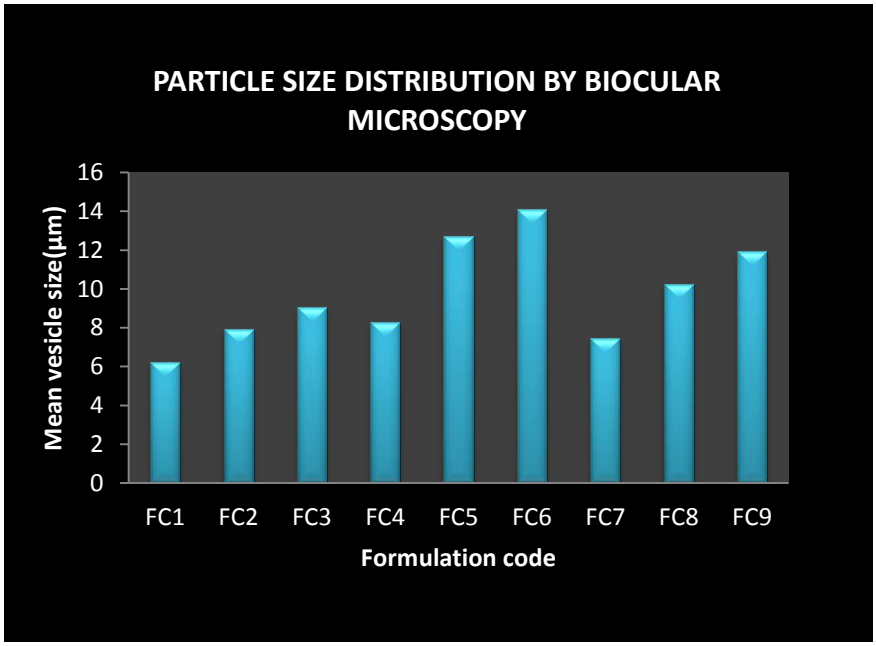
Table 24:Formulation table

FORMULATION CODE	NON – IONIC SURFACTANT	DRUG (mg)	LECITHIN: SURFACTANT: CHOLESTEROL (mg)
FC1	SPAN 40	300	100:100:100
FC2		300	100:200:100
FC3		300	100:300:100
FC4	SPAN 60	300	100:100:100
FC5		300	100:200:100
FC6		300	100:300:100
FC7	TWEEN 60	300	100:100:100
FC8		300	100:200:100
FC9		300	100:300:100

Table 25: Mean vesicle size, drug content and entrapment efficiency of lamivudine proniosomes by coacervation phase separation method

FORMULATION CODE	NON – IONIC SURFACTANT	MEAN VESICLE SIZE (µm)	% DRUG CONTENT (% w/w)	% ENTRAPMENT EFFICIENCY (% w/w)
FC1	SPAN 40	6.24	87.94	33.68
FC2		7.95	97.26	35.78
FC3		9.06	84.68	51.51
FC4	SPAN 60	8.25	95.86	51.34
FC5		12.69	97.26	63.06
FC6		14.13	90.74	**77.89**
FC7	TWEEN 60	7.47	87.48	50.38
FC8		10.23	93.08	60.39
FC9		11.94	73.02	73.78

Figure 26: Particle size distribution of Lamivudine proniosome gel



Lamivudine proniosome gel prepared using Span 40, Span 60 and Tween 60 at various concentrations . The particle size of the proniosomes gel was measured using an binocular microscope with calibrated eyepiece micrometer. From every batch about 100 vesicle were measured for the individually and the average diameter was calculated. The average vesicular size of hydrated proniosomes was in the range of 6.24 to 9.06 µm for FC1 to FC3; 8.25 to 14.13 µm for FC4 to FC6 and 7.47 to 11.94 µm for FC7 to FC9 formulations. The size distribution was in the range of 6 to 15 µm. The result suggests that proniosomes prepared were of uniform size and spherical in shape. The microphotographs of all hydrated proniosomal formulations reveal that the niosomes were spherical in their shape. From these results the vesicle size were found to be increase with increase in the concentration of surfactant [40].

Figure 27: Drug Content of Lamivudine proniosome gel

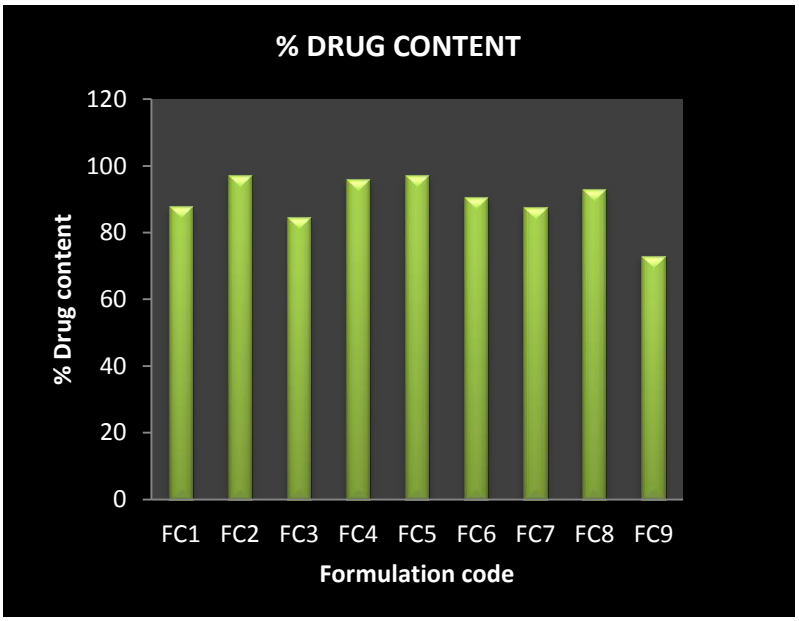
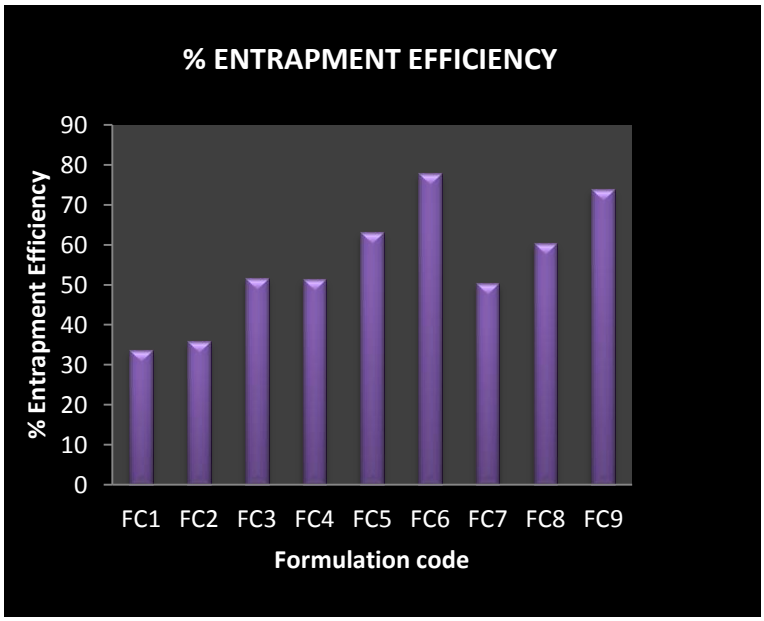


Figure 28: Entrapment Efficiency of Lamivudine proniosome gel



The entrapment efficiency of proniosomes gel prepared at various concentration of surfactants . The percentage entrapment efficiency was found to be in the range of 33.68 to 51.51 for FC1 to FC3; 51.34 to 77.89 for FC4 to FC6; 50.38 to 73.78 for FC7 to FC9 formulations. Higher surfactant concentration of Span 60, Span 40 and Tween 60 showed higher entrapment efficiency which might be due to the high fluidity of the vesicles. The entrapment efficiency was increased with increasing surfactant concentration. The larger vesicle size may also contribute to the higher entrapment efficiency. The highest entrapment efficiency was observed with Span 60 (FC6) . This may be due to the high phase temperature of Span 60 compared to Span 40 and Tween 60 . This results coinside with earlier reports [42,46,48].

The order of non ionic surfactants that resulted in better entrapment efficiency was as follows:

Span 60> Tween 60> Span 40

IN- VITRO RELEASE STUDY

The *in- vitro* release study of Lamivudine proniosomes gel was carried out using dialysis membrane in an open ended tube- beaker assembly using distilled water as the diffusion medium.

Table 26: *In- vitro* release of proniosomes containing Span 40 in different ratios

Time in hours	Cumulative % drug release			
	Control	**FC1**	**FC2**	**FC3**
0	0	0	0	0
0.5	-	10.58	5.91	8.45
1	64.23	12.53	7.21	9.59
1.5	-	12.95	9.25	11.31
2	85.53	15.11	12.35	12.91
2.5	-	17.98	11.25	14.91
3	105.5	22.16	13.81	16.17
3.5	-	19.66	16.38	19.77
4	-	20.43	19.03	22.28
4.5	-	21.12	20.88	23.46
5	-	22.51	22.26	24.86
6	-	25.06	24.83	27.06
7	-	25.68	26.21	30.06
8	-	24.06	29.81	31.95
9	-	28.53	33.01	34.65
10	-	29.93	37.35	37.00
11	-	31.26	41.66	39.77
12	-	33.86	43.15	42.78
24	-	39.91	47.63	**49.73**

Table 27: *In- vitro* release of proniosomes containing Span 60 in different ratios

Time in hours	Cumulative % drug release			
	Control	**FC4**	**FC5**	**FC6**
0	0	0	0	0
0.5	-	8.81	6.51	8.25
1	64.23	9.76	8.83	10.49
1.5	-	12.28	12.41	13.28
2	85.53	14.85	15.41	16.04
2.5	-	17.47	18.41	18.68
3	105.5	18.97	21.29	21.37
3.5	-	21.49	23.45	23.94
4	-	23.06	25.47	26.47
4.5	-	24.81	29.02	28.54
5	-	25.68	30.88	31.56
6	-	28.80	34.02	35.46
7	-	30.31	36.96	38.84
8	-	32.17	40.86	44.37
9	-	34.48	43.00	51.99
10	-	37.82	45.09	57.74
11	-	40.05	46.61	62.85
12	-	44.81	49.15	66.87
24	-	53.16	58.39	**72.44**

Table 28: *In- vitro* release of proniosomes containing Tween 60 in different ratios

Time in hours	Cumulative % drug release			
	Control	**FC7**	**FC8**	**FC9**
0	0	0	0	0
0.5	-	7.09	7.41	7.61
1	64.23	7.81	9.31	8.91
1.5	-	12.13	12.65	10.01
2	85.53	13.41	14.65	11.85
2.5	-	15.23	17.43	13.61
3	105.5	17.85	18.35	15.10
3.5	-	19.15	21.03	17.12
4	-	22.04	24.33	19.19
4.5	-	24.20	26.53	21.04
5	-	25.80	27.61	24.75
6	-	27.63	30.85	29.70
7	-	29.67	32.15	34.91
8	-	32.53	33.15	39.96
9	-	33.49	37.21	45.56
10	-	36.98	38.18	50.49
11	-	38.78	39.66	54.06
12	-	40.45	41.08	58.51
24	-	45.37	51.26	**67.70**

Figure 29: *In- vitro* release of proniosomes containing Span 40 in different ratios

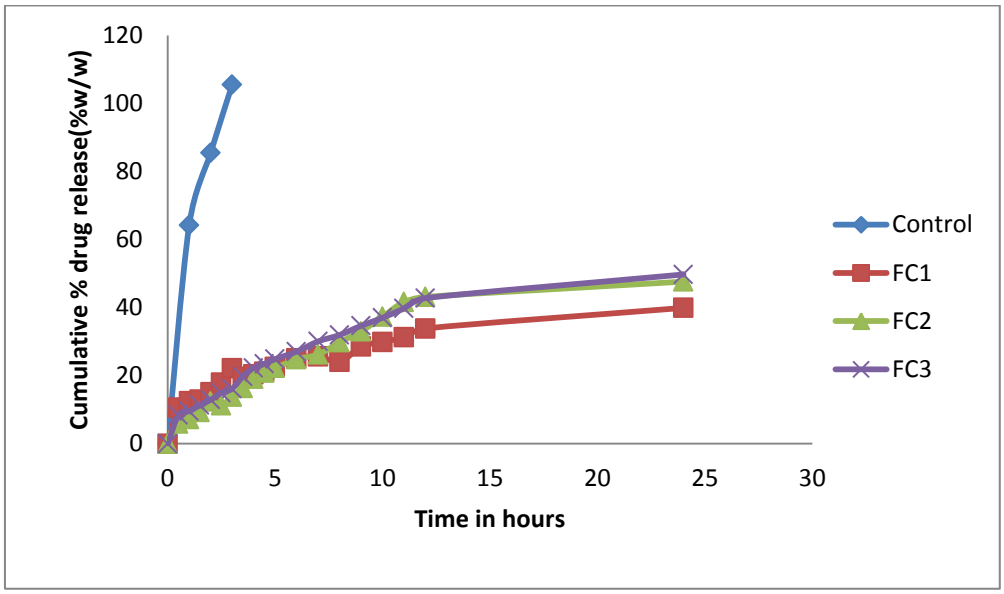


Figure 30: *In- vitro* release of proniosomes containing Span 60 in different ratios

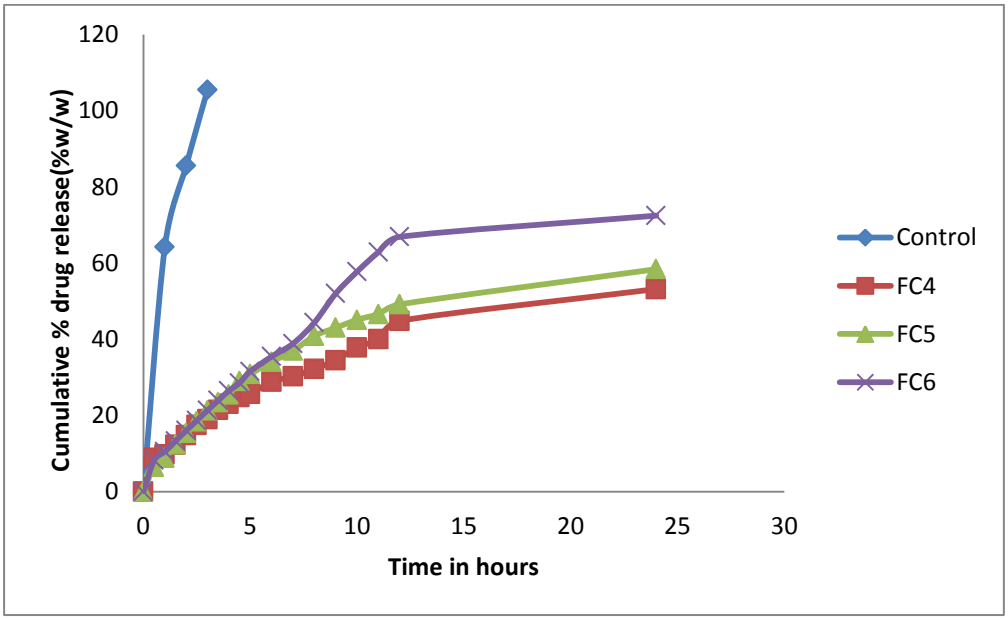


Figure 31: *In- vitro* release of proniosomes containing Tween 60 in different ratios

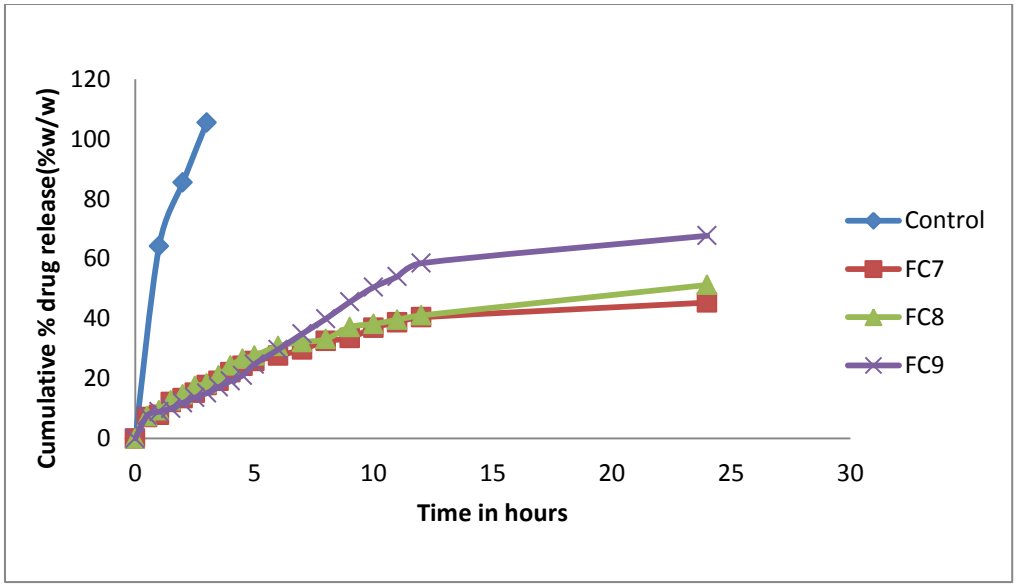
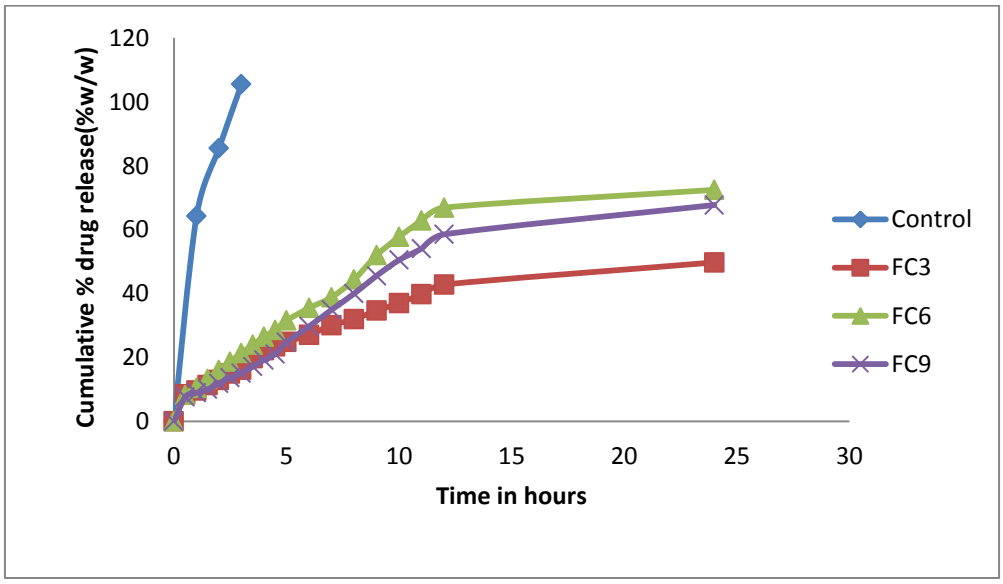


Figure 32: Comparison of *in- vitro* release of proniosomes with highest entrapment efficiency



The *in-vitro* release of Lamivudine proniosomes gel were prepared at various concentration of surfactants. The cumulative percentage of drug release was found to be in the range of 39.91 to 49.73 for FC1 to FC3; 53.16 to 72.44 for FC4 to FC6; 45.37 to 67.70 for FC7 to FC9 formulations. Higher surfactant concentration of Span 60, Span 40 and Tween 60 showed higher entrapment efficiency and more sustained release. The more sustained drug release was observed with Span 60 (FC6) . This may be due to the higher entrapment efficiency. The FC6 formulation showed sustained release of 72.44% over 24 hours. This results coinside with earlier reports[46,48].

All the proniosomal formulations showed sustained release when compared with control.

The cumulative % drug release at 24 hours for different ratios of the three non- ionic surfactants was in the following order:

Span 40 : FC1>FC2>FC3

Span 60 : FC4>FC5>FC6

Tween 60 : FC7>FC8>FC9

COMPARISION OF OF LAMIVUDINE PRONIOSOMES PREPARED BY SLURRY AND COACERVATION PHASE SEPARATION METHODS

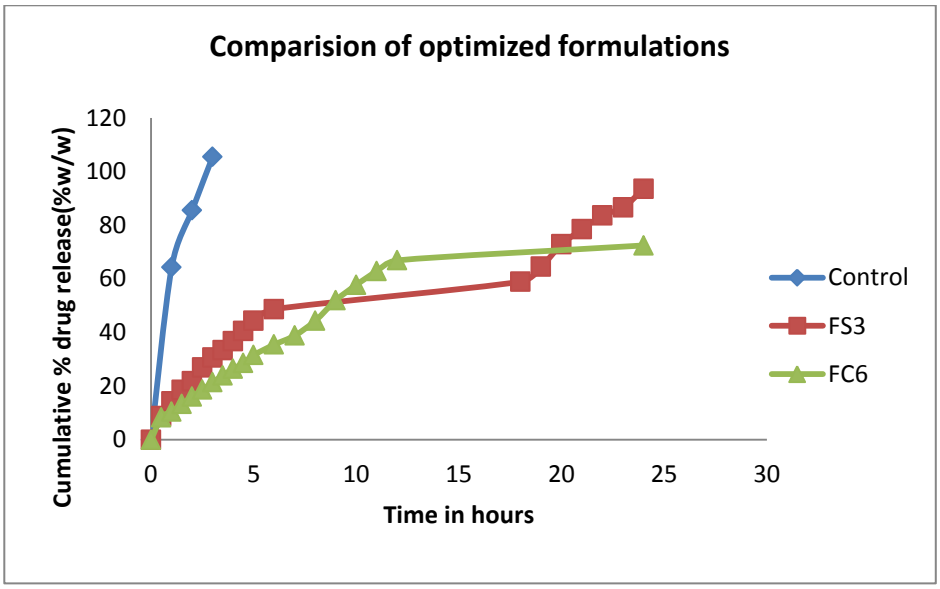
Table 29: Comparision of entrapment efficiency of Lamivudine proniosomes

Non – Ionic Surfactant	Formulation Code	% Entrapment Efficiency (% W/W)	Formulation Code	% Entrapment Efficiency (% W/W)
SPAN 40	FS1	76.24	FC1	33.68
	FS2	71.13	FC2	35.78
	FS3	**95.02**	FC3	51.51
SPAN 60	FS4	82.05	FC4	51.34
	FS5	83.33	FC5	63.06
	FS6	85.54	FC6	**77.89**
TWEEN 60	FS7	45	FC7	50.38
	FS8	55.86	FC8	60.39
	FS9	86.85	FC9	73.78

Table 30: Comparision of *in-vitro* drug release (at 24 th hour) of lamivudine proniosomes

Non – Ionic Surfactant	Formulation Code	Cumulative % drug release (% W/W)	Formulation Code	Cumulative % drug release (% W/W)
SPAN 40	FS1	-	FC1	39.91
	FS2	-	FC2	47.63
	FS3	**93.53**	FC3	49.73
SPAN 60	FS4	-	FC4	53.16
	FS5	-	FC5	58.39
	FS6	-	FC6	**72.44**
TWEEN 60	FS7	-	FC7	45.37
	FS8	-	FC8	51.26
	FS9	-	FC9	67.70

Table 33: Comparision of *in-vitro* drug release of optimized lamivudine proniosomes



The cumulative percentage of drug release of the optimized formulation of Lamivudine proniosome powder (FS3) and proniosome gel (FC6) were compared. From these results the FS3 formulation showed more sustained release over 24 hours when compared to FC6 formulation due to the higher entrapment efficiency of FS3 formulation (95.02%).

EFFECT OF SURFACTANT CONCENTRATION ON VESICLE SIZE

- The vesicle size of the hydrated proniosome powder and gel was measured using a binocular microscope with calibrated eyepiece micrometer. From every batch about 100 vesicles were measured individually and then average diameter was calculated.
- The result suggests that hydrated proniosome powder and gel were of uniform size. The microphotographs of all proniosomal formulations reveal that the niosomes were spherical in their shape.
- From these results the vesicle size was found to increase with increase in the concentration of surfactant.

Among the formulations prepared by two methods using Span 40 the vesicle size were found to increase with increase in the concentration of surfactant .The increase in vesicle size was found to be in the following order

FS1>FS2>FS3

FC1>FC2>FC3

Among the formulations prepared by two methods using Span 60 the vesicle size were found to increase with increase in the concentration of surfactant . The increase in vesicle size was found to be in the order

FS4>FS5>FS6

FC4>FC4>FC6

Among the formulations prepared by two methods using Tween 60 the vesicle size were found to increase with increase in the concentration of surfactant. The increase in vesicle size was found to be in the order

FS7>FS8>FS9

FC7>FC8>FC9

EFFECT OF SURFACTANT CONCENTRATION ON ENTRAPMENT EFFICIENCY

- The Lamivudine proniosomes were prepared at various surfactant concentrations.
- Higher surfactant concentration of span 60, span 40 and Tween 60 showed higher entrapment efficiency which might be due to the high fluidity of the vesicles [27].
- The entrapment efficiency increase with increasing surfactant concentration.

Among the formulations prepared by two methods using Span 40 the % entrapment efficiency were found to increase with increase in the concentration of surfactant. The increase in % entrapment efficiency was found to be in the order of

FS1>FS2>FS3

FC1>FC2>FC3

Among the formulations prepared by two methods using Span 60 the % entrapment efficiency were found to increase with increase in the concentration of surfactant. The increase in % entrapment efficiency was found to be in the order of

FS4>FS5>FS6

FC4>FC4>FC6

Among the formulations prepared by two methods using Tween 60 the% entrapment efficiency were found to increase with increase in the concentration of surfactant. The increase in % entrapment efficiency was found to be in the order of

FS7>FS8>FS9

FC7>FC8>FC9

EFFECT OF SURFACTANT CONCENTRATION ON DRUG RELEASE

- The Lamivudine proniosomes were prepared at various concentration of surfactants.
- Higher surfactant concentration of Span 60, Span 40 and Tween 60 showed more sustained release due to higher entrapment efficiency.
- The cumulative percentage drug release more sustained with increasing the concentration of surfactant.

Among the formulations prepared by two methods using Span 40 the cumulative % drug release were found to increase with increase in the concentration of surfactant. The increase in % drug release was found to be in the order

FS1>FS2>FS3

FC1>FC2>FC3

Among the formulations prepared by two methods using Span 60 the cumulative % drug release were found to increase with increase in the concentration of surfactant. The increase % drug release was found to be in the order

FS4>FS5>FS6

FC4>FC4>FC6

Among the formulations prepared by two methods using Tween 60 the cumulative % drug release were found to increase with increase in the concentration of surfactant. The increase in the cumulative % drug release was found to be in the order

FS7>FS8>FS9

FC7>FC8>FC9

KINETICS OF DRUG RELEASE

The *in- vitro* release data was applied to various kinetic models to predict the mechanism of drug release of the optimized formulation **FS3**

Table 31: Kinetics Data

Time in hours	% cumulative drug release	Log % cumulative drug release	Sq.root of time	Log time	% drug remaining	Cube root of drug remaining	Log % drug remaining
0	0	-∞	0	-∞	100	4.64	2
0.5	8.8	0.944	0.707	-0.301	91.21	4.501	1.959
1	14.22	1.152	1	0	85.78	4.410	1.933
1.5	18.53	1.267	1.224	0.176	81.42	4.334	1.910
2	21.82	1.338	1.414	0.301	78.59	4.283	1.895
2.5	26.90	1.429	1.581	0.397	73.11	4.181	1.863
3	30.58	1.485	1.732	0.477	69.42	4.109	1.841
3.5	33.35	1.523	1.870	0.544	66.65	4.054	1.823
4	36.69	1.564	2	0.602	63.31	3.985	1.801
4.5	40.55	1.607	2.121	0.653	59.45	3.902	1.774
5	44.39	1.647	2.236	0.698	55.61	3.816	1.745
6	48.68	1.687	2.449	0.778	51.32	3.716	1.710
18	58.98	1.770	4.242	1.255	41.06	3.449	1.613
19	64.41	1.808	4.358	1.278	35.53	3.287	1.550
20	72.46	1.862	4.472	1.301	27.54	3.005	1.433
21	78.46	1.894	4.582	1.322	21.54	2.782	1.333
22	83.65	1.922	4.690	1.342	16.35	2.538	1.213
23	86.66	1.937	4.795	1.361	13.34	2.371	1.125
24	93.53	1.970	4.898	1.380	6.47	1.863	0.810

Figure 34: Zero- Order Release Kinetics of FS3

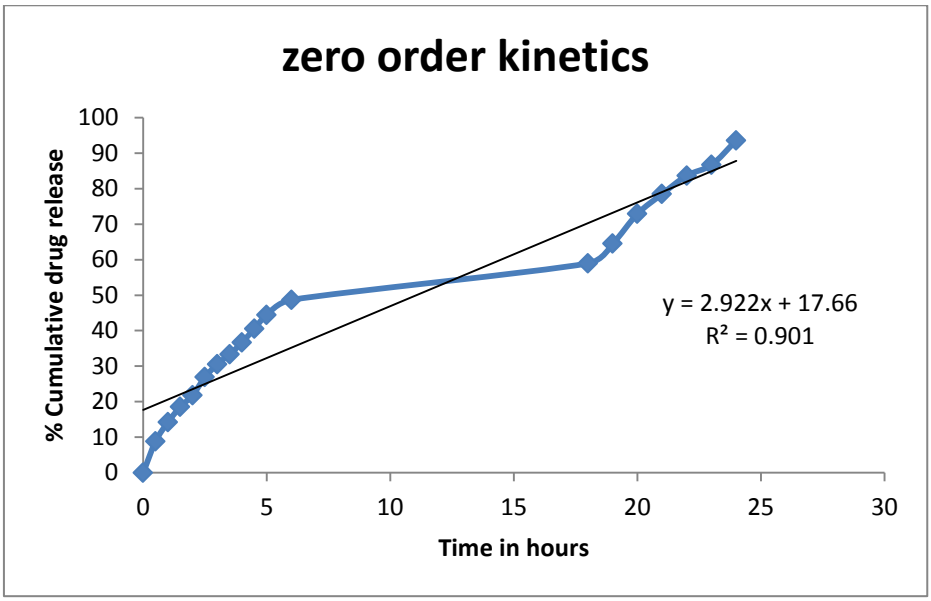


Figure 35: First Order Release Kinetics of FS3

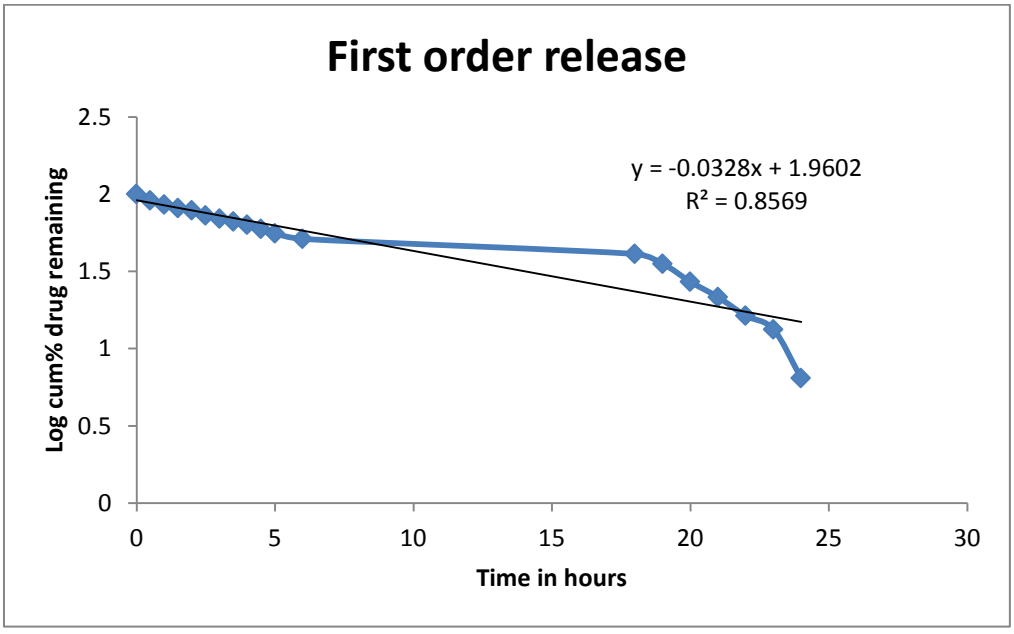


Figure 36: Higuchi Model Kinetics of FS3

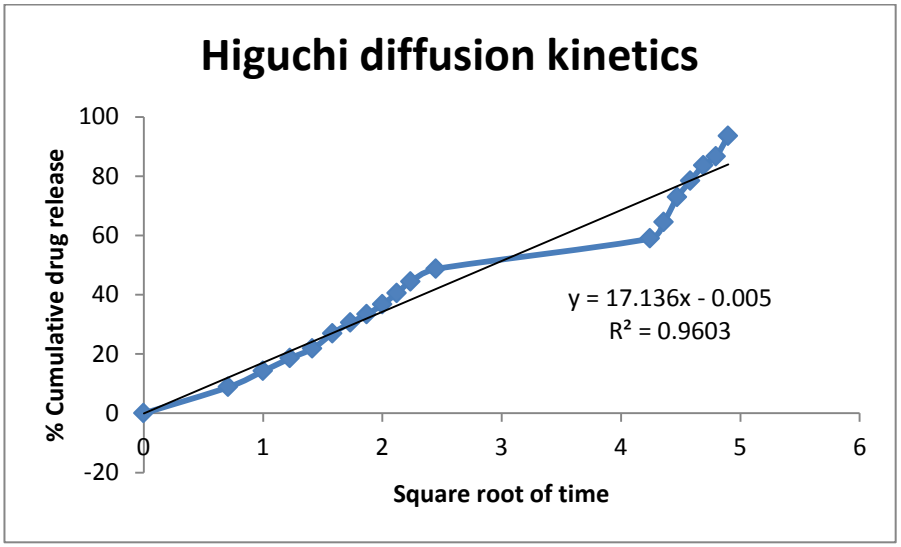


Figure 37: Hixson Crowell Model Kinetics of FS3

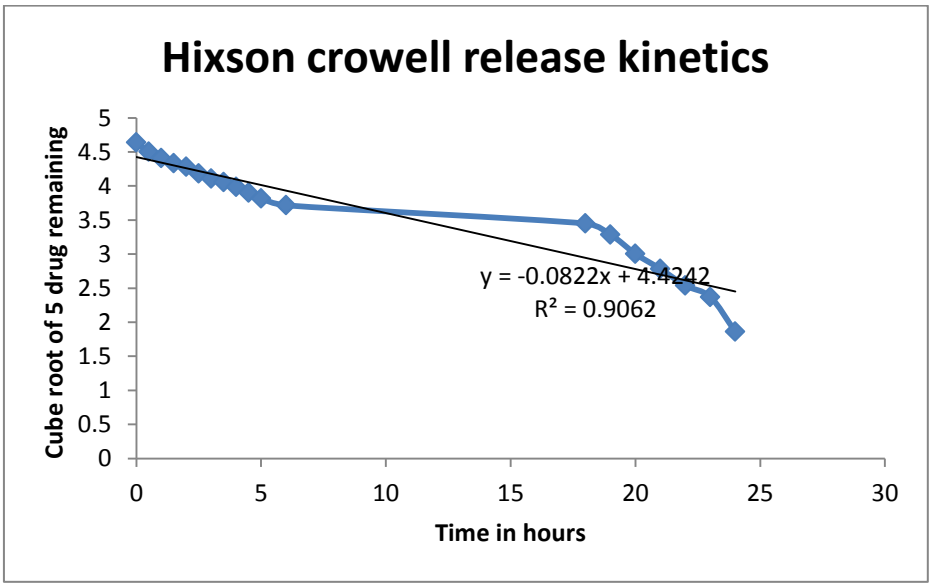
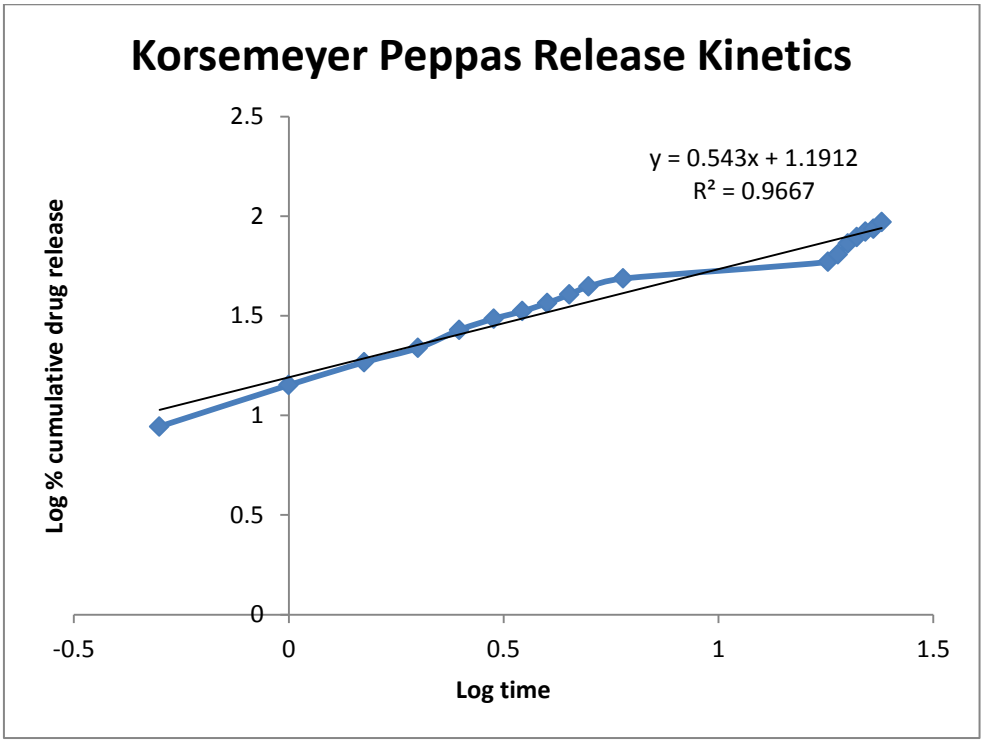


Figure 38: Korsemeyer- Peppas Model Kinetics of FS3



The regression coefficient (r^2) was determined using the drug release data. The results are shown in Table 32.

Table 32: Drug Release Kinetics

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI MODEL	KORSEMEYER PEPPAS		HIXSON CROWELL
	R^2	R^2	R^2	R^2	n	R^2
FS3	0.901	0.856	0.960	0.966	0.543	0.906

The drug release pattern from Lamivudine loaded Proniosome (FC3) follows **Korsemeyer peppas and Higuchi model.** The value of the krosemeyer peppas model for FS3 was found to be 0.543 . Which confirm the Non-fickian type diffusion (or) anamalous diffusion mechanism [27].

STABILITY STUDIES OF LAMIVUDINE PRONIOSOMES

Stability studies of the optimized formulation FS3 were carried out by storing at 4 ^{0}C ± 2 ^{0} C (refrigeration temperature) and 25^{0}C ± 2 ^{0}C for a period of 45 days.

Table 33: Stability Study of Lamivudine Proniosomes – Optimized Formulation FS3

Time of storage in days	**Temperature of storage**			
	4 ^{0}C ± **2** ^{0} C (Refrigerator temperature)		**4** ^{0}C ± **2** ^{0} C (Room temperature)	
	Drug content(%)	**Entrapment efficiency(%)**	**Drug content(%)**	**Entrapment efficiency(%**
0	**90.67**	**95.02**	**90.67**	**95.02**
15	**90.50**	**94.52**	**88.53**	**93.21**
30	**90.22**	**94.11**	**87.20**	**90.32**
45	**89.91**	**93.72**	**84.31**	**89.81**

Figure 39 :Stability of optimized formulation % drug content analysis

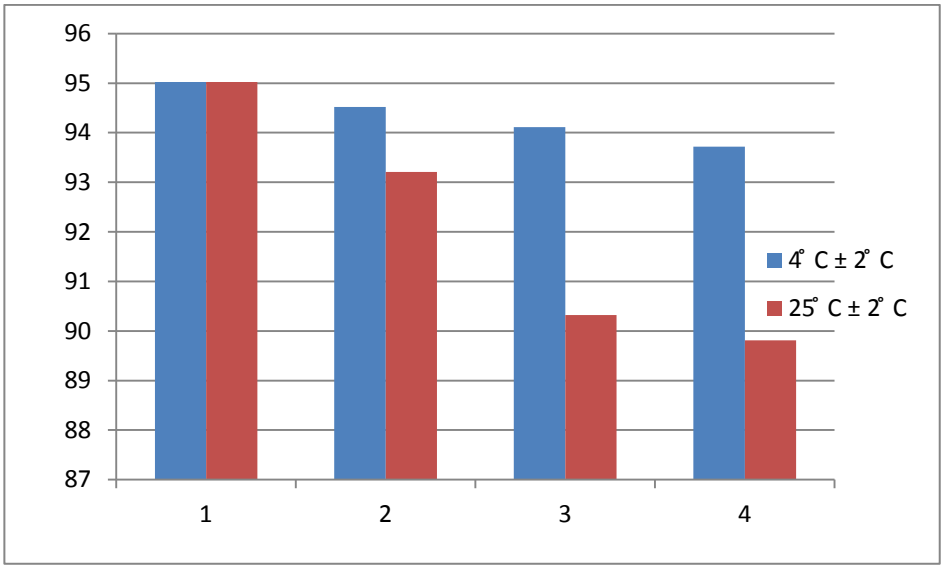
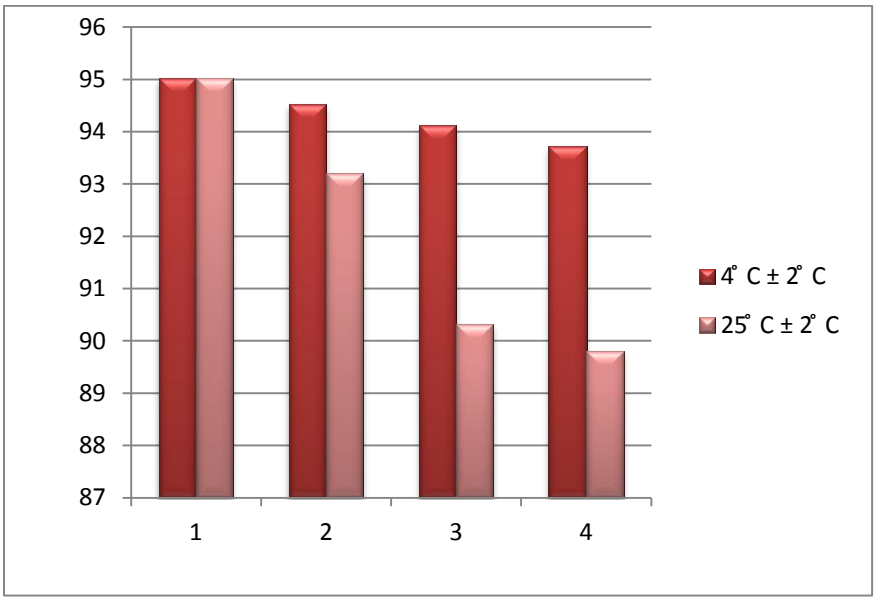


Figure 40:Stability of optimized formulation % Entrapment efficiency



Stability studies of the optimized formulation FS3 were carried out by storing at **4** ^{0}C ± **2** ^{0}C (refrigeration temperature) and **4** ^{0}C ± **2** ^{0}C for a period of 45 days. The drug leakage from the vesicles was least at 4^0 C. This may be attributed to phase transition of non-ionic surfactant and lipid causing leakage of vesicles at higher temperatures during storage. Hence the proniosomes can be stored at 4 ^{0} C. This results coinside with earlier reports [10,30].

SCANNING ELECTRON MICROSCOPY

SEM image of optimized proniosomal formulations were recorded. The particles are almost spherical and homogeneous. The results showed that the Lamivudine loaded proniosomes have a spherical shape with smooth surface and discrete without any aggregation or agglomeration.

Figure 41: Optimized Lamivudineproniosome powder (FS3)

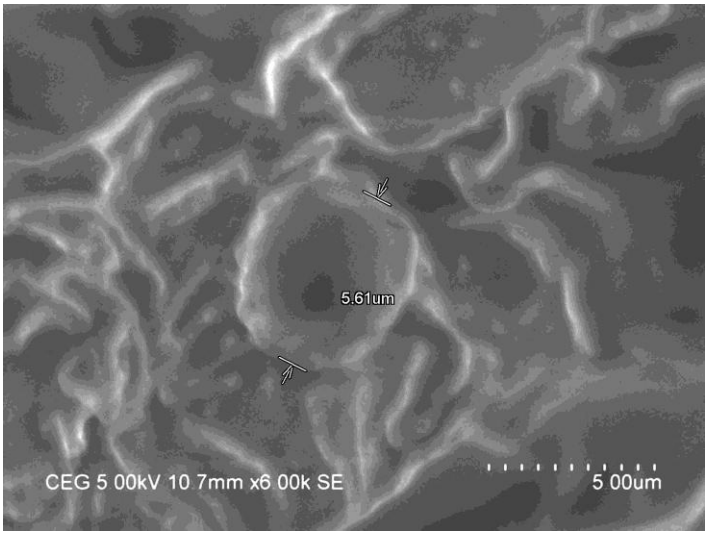


Figure 42: Photomicroscopy of FS3

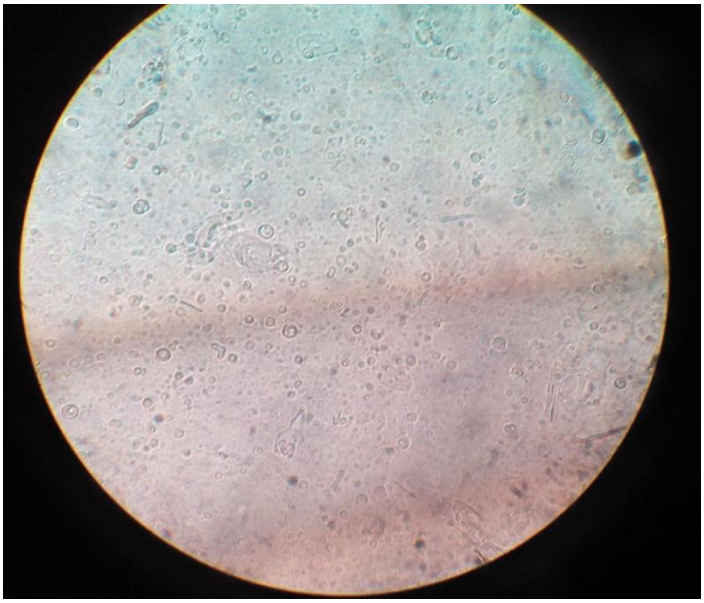


Figure 43: Optimized Lamivudine proniosome gel (FC6)

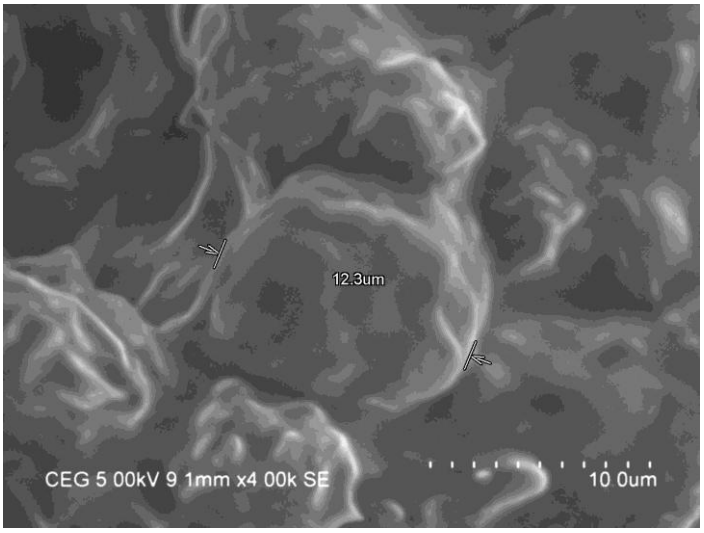
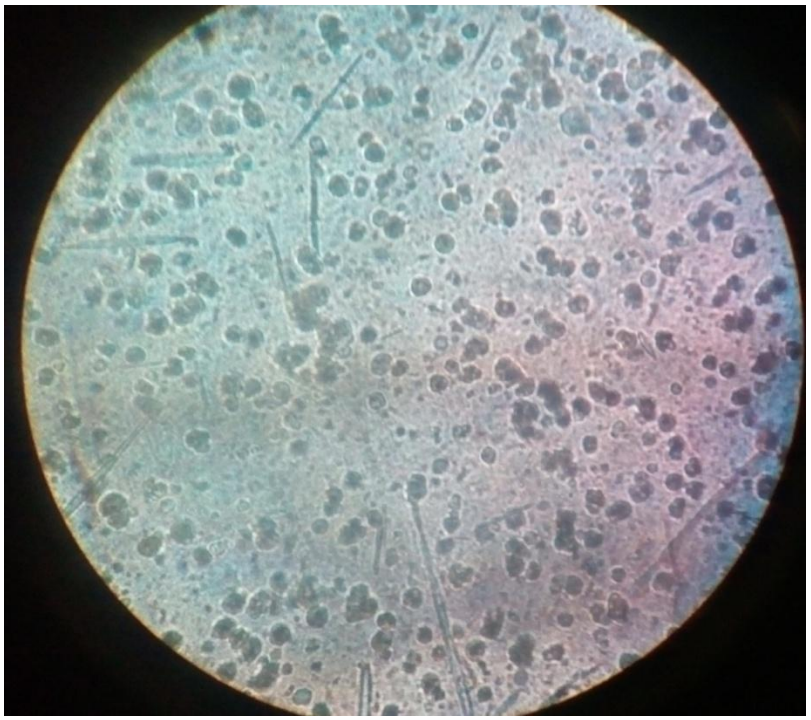


Figure 44: Photomicroscopy of FC6



10. SUMMARY AND CONCLUSION

- ✓ The purpose of this research was to formulate Lamivudine proniosome drug delivery system by coacervation phase separation and slurry method using different non-ionic surfactants.
- ✓ The formulations were prepared using different non ionic surfactants in various the surfactant concentrations (Span 40, Span 60 and Tween 60) keeping the concentration of Cholesterol, Maltodextrin, Lecithin as constant.
- ✓ The prepared Lamivudine powder by slurry and Lamivudine gel by coacervation phase separation method were characterized for entrapment efficiency and mean vesicle diameter (MVD).
- ✓ The percentage drug entrapment of Lamivudine powder ranged from **45 to 95.02%.**
- ✓ The percentage drug entrapment of Lamivudine gel ranged from **33.68 to 77.89%.**
- ✓ The cumulative percentage of drug release at (24^{th} hour) of optimized Lamivudine powder was (FS3) **93.53%** and optimized Lamivudine gel was (FC6) **72.44%** .
- ✓ From the resultant entrapment efficiency and *in-vitro* drug release data, slurry method is better than coacervation method to formulate Lamivudine proniosomes.
- ✓ The order of entrapment efficiency of the proniosome powder.

  Span 40 > Span 60> Tween 60
- ✓ The order of entrapment efficiency of the proniosome gel

  Span 60 > Tween 60 > Span 40
- ✓ The order of *in vitro* drug release of the proniosome powder

  Span 40 > Span 60>Tween 60
- ✓ The order of *in vitro* drug release of the proniosome gel

  Span 60 > Tween 60 > Span 40
- ✓ From these resulat sustained drug release strongly influenced by entrapment efficiency.
- ✓ From all these formulations FS3 prepared by slurry method was optimized which had better entrapment efficiency **(95.02%)** and better sustained release over 24 hours **(93.53%).**

- ✓ Effect of surfactant concentration on vesicle size was studied. The vesicle size increased with increase in the surfactant concentration.
- ✓ Effect of surfactant concentration on entrapment efficiency was studied. The entrapment efficiency increased with increase in the surfactant concentration.
- ✓ Effect of surfactant concentration on rate of drug release was studied. The more sustained drug release was observed with increase in the surfactant concentration.
- ✓ SEM analysis of the optimized proniosome powder (FS3) showed the spherical shape of the vesicles.
- ✓ The drug release pattern from Lamivudine loaded Proniosome (FC3) follows **Korsemeyer peppas and Higuchi model .**
- ✓ The results of FT-IR studies proved that there is no interaction between the drug and Excipients.
- ✓ From the stability studies, the optimum storage condition for Lamivudine proniosome was found to be $4^{\circ}C$.

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FUTURE SCOPE

- ✓ The Lamivudine proniosome powder formulation may be designed into suitable delivery systems such as tablet, capsule forms.
- ✓ The Lamivudine proniosome gel formulation may be designed into suitable delivery systems such as transdermal form.

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